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THE CLINICAL USE OF PHTHALYLSULFATHIAZOLE

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AN EXTENSIVE study covering twenty acylated sulfonamides[1, 2] in an attempt to find substances possessing antibacterial properties and being poorly absorbed from the alimentary tract has resulted in the synthesis and discovery of several compounds fulfilling these specifications. The first of these drugs of therapeutic value, succinylsulfathiazole, was described in 1941 by Poth and Knotts,[3] having been synthesized by Miller, Rock and Moore.[4]

This compound when administered by mouth profoundly alters the bacterial flora of the gastrointestinal tract and the physical character of the stools. The use of this drug[5] in the preoperative preparation of patients due for operations upon the large bowel brought the individuals to operation in excellent physical condition with the bowel emptied of fecal contents and nicely prepared for surgical treatment. The simplification of the bacterial flora of the gastrointestinal tract by the significant reduction of and by the frequent elimination of the coliform organisms, the *Clostridia,* the bacteroides, and numerous other microorganisms offers considerable protection against the development of a fatal peritonitis, especially as emphasized by the studies of Meleny, Olpp, Harvey and Zaytseff-Jern (1932)[6] who demonstrated that the intraperitoneal inoculation of mixtures of *B. coli, B. welchii,* and alpha streptococci was ten to fifteen times as lethal as pure cultures of these organisms. Postoperatively, the patients treated preoperatively with a drug like succinylsulfathiazole do well, there having resulted no instances of peritonitis or locally detectable infection, although operations were often performed on the open colon. These patients experienced a smooth convalescence with minimal gaseous distention and gas pains.

Succinylsulfathiazole has been used for the treatment of bacillary dysentery with excellent results first by Poth, Chenoweth, and Knotts (1942)[7] and subse-

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quently by numerous investigators. The most significant investigation having been reported by Hardy, Burns, and De Capito[8] who showed it to be the most effective of the sulfonamides in preventing the development of carriers.

The toxicity of succinylsulfathiazole is particularly low and is probably the least toxic of all the effective sulfonamides which have been used extensively. But, it should be repeatedly emphasized that all members of this series of drugs can be expected to show toxic reactions and that these reactions may indeed be serious if administered to a sensitized or highly susceptible individual. The incidence of the more severe reactions is very low for succinylsulfathiazole. A single instance of agranulocytosis with a fatal outcome has been reported by Johnson (1943).[9, 10] It is not entirely clear that this complication was due to the action of succinylsulfathiazole although it is true that the individual had previously been shown to have been sensitive to sulfathiazole.

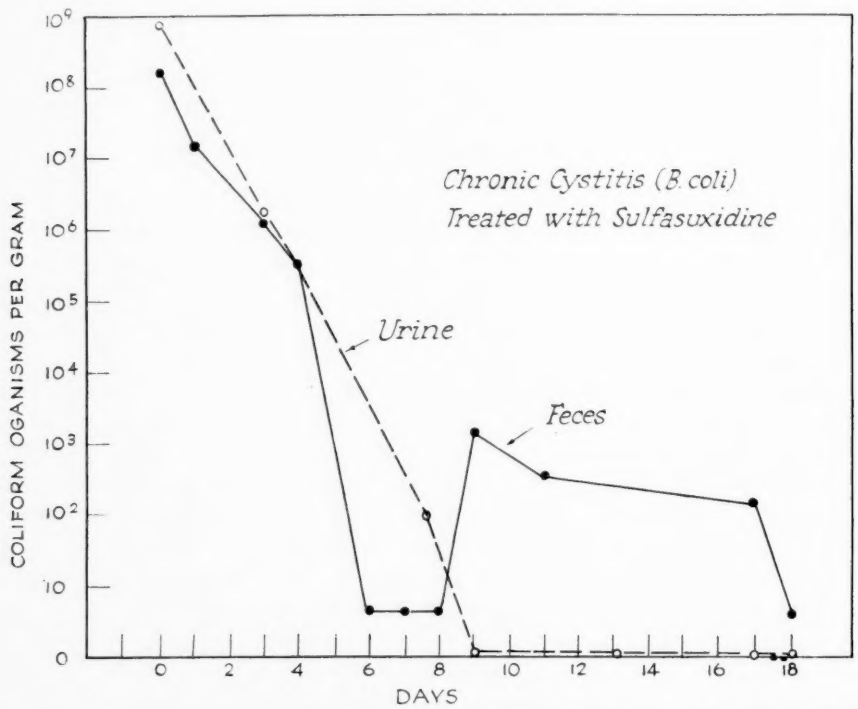

Chart 1.—Demonstration of rapid disappearance of *B. coli* from the urine of a patient with a long existing chronic cystitis. The urine became sterile between the third and ninth days. No coliform organisms present in the feces on the sixth day. The patient received 0.25 Gm. of succinylsulfathiazole daily divided into six equal doses administered orally at four-hour intervals.

The following patient presents a particularly interesting therapeutic application of succinylsulfathiazole. An 82-year-old woman gave a history of abdominal distention and discomfort daily over a period of ten years. She had been troubled with constipation most of her life. There had been recurring attacks of pyelitis and cystitis. When first seen two years ago, she was suffering from an unusually severe urinary tract infection and a moderate anemia and leucopenia were present. There were 1,000,000 *B. coli,* per cubic centimeter in the urine. Under treatment with succinylsulfathiazole, the coliform organisms in the feces and urine were reduced as indicated in Chart 1. The urine became

sterile. The drug was discontinued after three weeks. The constipation and flatulence were relieved. After one month, evidence of a cystitis returned and the urine again contained *B. coli.* Immediate response to succinylsulfathiazole therapy occurred again. After this sequence of events had repeated itself several times and upon the insistence of the patient, and, since the hemoglobin content of the blood had increased and the leucocyte count had improved, 0.125 Gm. of succinylsulfathiazole per kilogram of body weight divided into three daily doses were administered for one week each month. This regimen has kept the patient free of cystitis, flatulence and constipation and no untoward reactions have occurred during a period of two years.

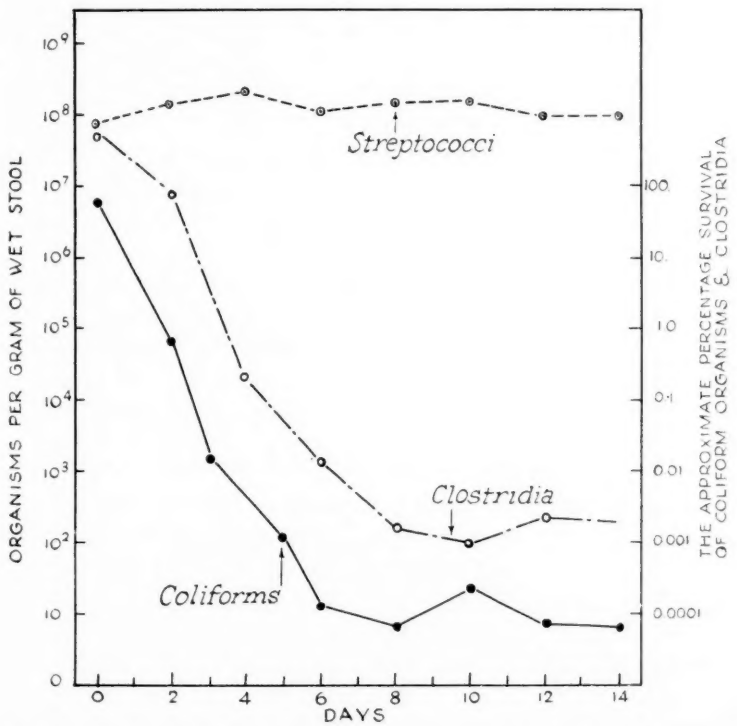

Chart 2.—An illustration of the variation of different bacterial groups following the oral administration of 0.0833 Gm. of phthalylsulfathiazole per kilo of body weight every four hours to dogs on a submaintenance diet of boiled meat. The streptococci are not affected. The coliform organisms and *Clostridia* are reduced in number in a regular manner. The counts for the *Clostridia* represent the sum of both the vegetative forms and spores, and it is interesting to record that after two days' therapy the vegetative forms disappear with only the heat-resistant spores remaining. These data represent the averages of the observations made on two dogs. The individual observations agree within the limits of experimental error in all instances.

Poth and Ross (1943)[2, 11] reported experimental studies on phthalylsulfathiazole as an intestinal antiseptic. This compound, first synthesized by Moore and Miller (1942),[12] was shown to be from two to four times as effective as succinylsulfathiazole in altering the coliform flora in the alimentary tract of the dog. When phthalylsulfathiazole was administered orally to dogs every four hours in doses varying from 0.04 to 0.08 Gm. per kilogram of body weight, the alteration of the coliform flora in the feces was comparable to that observed when 0.166 Gm. of succinylsulfathiazole were similarly administered.

As indicated in Chart 2, phthalylsulfathiazole reduces the *Clostridia* as well as the coliform bacteria to significantly low populations in the feces. It is

interesting that after forty-eight hours all anaerobic vegetative organisms disappear with only heat resistant spores persisting. Similar to the other sulfonamides, phthalylsulfathiazole is ineffective against the alpha *Streptococcus fecalis*. This drug alters the physical character of the stools by reducing the fecal odor, but the feces are rendered less liquid than is the case following the administration of succinylsulfathiazole. This latter finding might be considered an indication that phthalylsulfathiazole would be more effective in the presence of diarrhea, and consequently, be better suited to the treatment of the dysenteries, and be a possible therapeutic agent against cholera and in the treatment of the highly fatal, epidemic, neonatal diarrheas.

Phthalylsulfathiazole is rapidly excreted in the urine of the dog, and the concentration of the drug in the blood has not been observed to exceed 3.0 mg. per 100 cubic centimeters following sustained oral administration of the compound in effective therapeutic doses. No toxic manifestations occurred following such administration of the compound. A single dose of twenty-five Gm. per kilogram of body weight of phthalylsulfathiazole given by stomach tube produced no toxic symptoms, and the concentration of the drug in the blood did not exceed 2.0 mg. per 100 cubic centimeters.

Dogs survived the intravenous administration twice daily of one gram of the sodium salt of phthalylsulfathiazole per kilogram of body weight, for periods of seven days, although vomiting occurred during and following the injections and the animals showed a moderate loss of weight. Intraperitoneal injection of a suspension of the drug is less well tolerated. The L.D. 50 is approximately 2.5 Gm. per kilogram of body weight in single doses intraperitoneally.

THE ADMINISTRATION OF PHTHALYLSULFATHIAZOLE TO MAN

On the basis of the evidence of bacteriostasis and low toxicity of phthalylsulfathiazole obtained by the authors[2, 11] and by Mattis, Benson & Koelle[13] on various laboratory animals, the administration of the drug to man was begun in July, 1942. First, the administration was to individuals with normal alimentary tracts and then to persons with various gastrointestinal lesions and disorders including carcinomata of the colon, ulcerative colitis, nonspecific diarrheas, and bacillary dysentery.

When phthalylsulfathiazole is given to individuals with normal gastrointestinal mucosa in doses varying from 0.05 to 0.25 Gm. per kilogram of body weight daily divided into six equal portions administered at four-hour intervals day and night, the alteration of the coliform bacteria occurs as indicated in Chart 3. Doses of phthalylsulfathiazole as small as 0.017 Gm. per kilogram every four hours may effect a significant lowering of the coliform population in the feces of normal individuals. The time required to cause this alteration may be somewhat more prolonged than when larger doses are given. The comparative effectiveness of phthalylsulfathiazole and succinylsulfathiazole is presented in Chart 4 and demonstrates phthalylsulfathiazole to possess, in this instance, twice the bacteriostatic action as that shown by succinylsulfathiazole.

The effect of spacing the frequency of dosage is studied by the daily oral administration of 0.0625, 0.125, and 0.25 Gm. of phthalylsulfathiazole, dividing the respective daily doses, in each instance, into six, three, and single portions and giving the drug at 4, 8, and 24-hour intervals. Charts 5, 6, and 7 show the

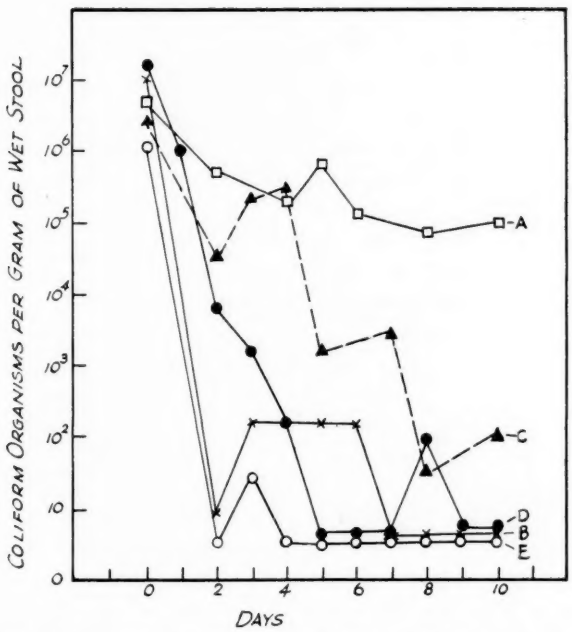

Chart 3.—The effect of various dosages of phthalylsulfathiazole on the coliform bacteria in the gastrointestinal tract of man. *A* represents the results after the oral administration of 0.05 Gm. per kilogram of body weight daily, divided into six equal portions and given at four-hour intervals; *B*, 0.0625 Gm. per kilogram; *C*, 0.10 Gm. per kilogram; *D*, 0.125 Gm. per kilogram; and *E*, 0.25 Gm. per kilogram.

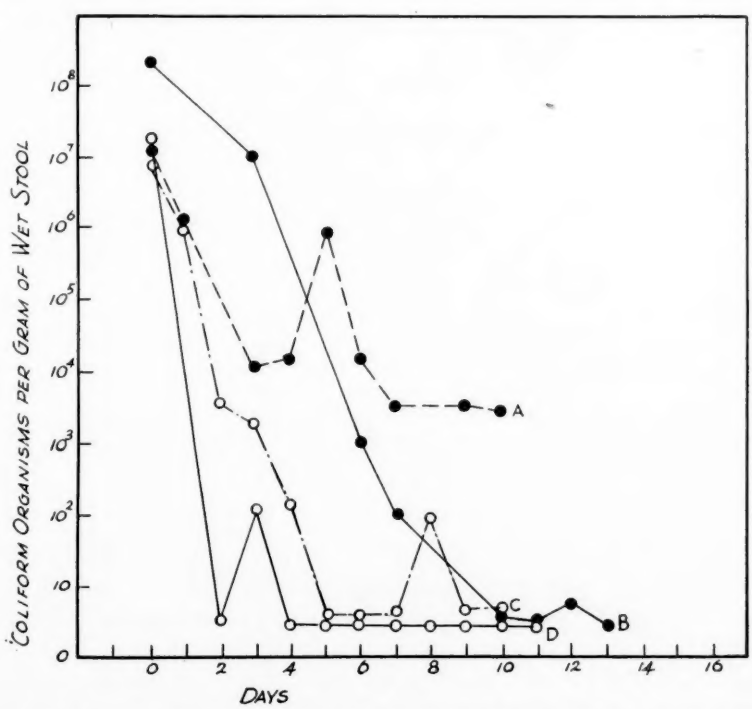

Chart 4.—A comparison of the relative bacteriostatic activity of succinylsulfathiazole and phthalylsulfathiazole as indicated by the alteration of the coliform flora in the gastrointestinal tract of man when equal doses of the drugs are administered orally at four-hour intervals. *A* represents the results following a daily dosage of 0.125 Gm. of succinylsulfathiazole per kilogram of body weight; *B*, following a daily dosage of 0.25 Gm. of succinylsulfathiazole; *C*, following a daily dosage of 0.125 Gm. of phthalylsulfathiazole; and *D*, following a daily dosage of 0.25 Gm. of phthalylsulfathiazole.

Compare *A* with *C*, and *B* with *D*.

alteration to be significant in each instance, but the rate of change and the magnitude of the change is decreased as the dosage is lowered and the interval of administration is lengthened. When the smallest dose, however, is given once daily, the coliform flora is lowered in count from 10^7 to $10,^4$ but even this change represents a disappearance of 99.9 per cent of the coliform bacteria initially present. These observations indicate that phthalylsulfathiazole may be better suited to prolonged administration as is required in the treatment of ulcerative colitis, because it need not be given at such frequent intervals. Likewise, it may find use as a prophylactic measure when it is known that an individual is exposed to acute bacillary infections such as dysentery and possibly cholera, as has been suggested previously by Poth, Chenoweth, and Knotts (1942).[7]

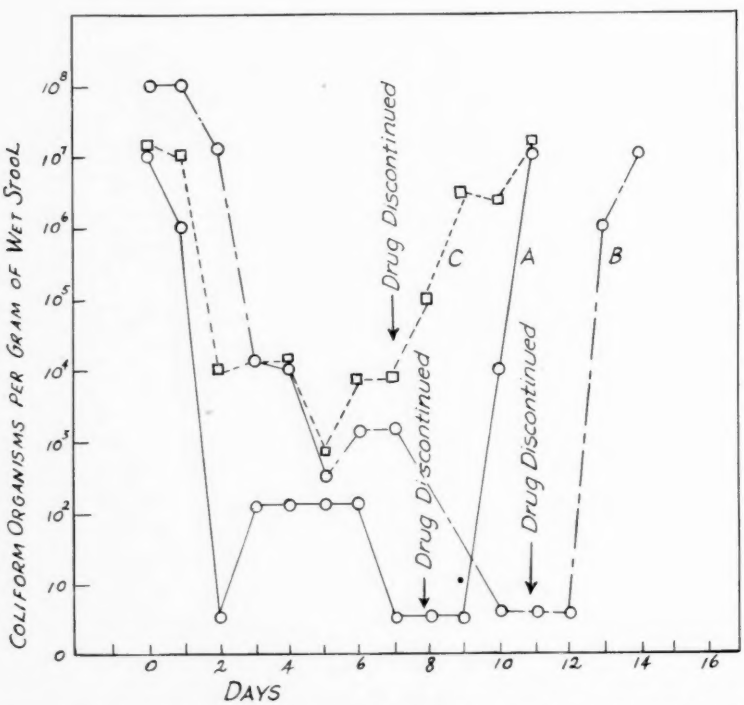

Chart 5.--The effect of spacing the oral dosage of 0.0625 Gm. of phthalylsulfathiazole per kilogram of body weight daily as indicated by the change in the number of coliform bacteria in the gastrointestinal tract of man when the drug is (*A*) divided into six equal doses and given at four-hour intervals; (*B*) divided into three equal doses and given at eight-hour intervals; and (*C*) given as a single daily dose.
Compare with Charts 6 and 7.

One of the drawbacks encountered in the administration of succinylsulfathiazole is that the drug tends to be ineffective in the presence of a watery diarrhea. Since the stools do not become as liquid following the administration of phthalylsulfathiazole as when the former drug is given and since phthalylsulfathiazole has the greater bacteriostatic activity, the value of the drug was studied in the presence of a watery diarrhea. An extremely watery diarrhea was produced and maintained for a period of two weeks by the administration of an ounce of magnesium sulfate twice daily to a healthy adult Negro male. Phthalylsulfathiazole, 0.125 Gm. per kilogram of body weight daily, divided

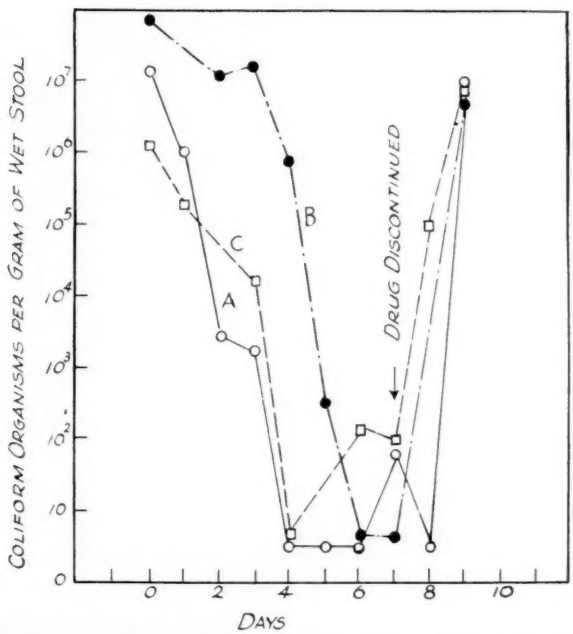

Chart 6.—The effect of spacing the oral dosage of 0.125 Gm. of phthalylsulfathiazole per kilogram of body weight daily, as indicated by the change in the number of coliform bacteria in the gastrointestinal tract of man, when the drug is (*A*) divided into six equal doses and given at four-hour intervals; (*B*) divided into three equal doses and given at eight-hour intervals; and (*C*) given as a single daily dose.
Compare with Charts 5 and 7.

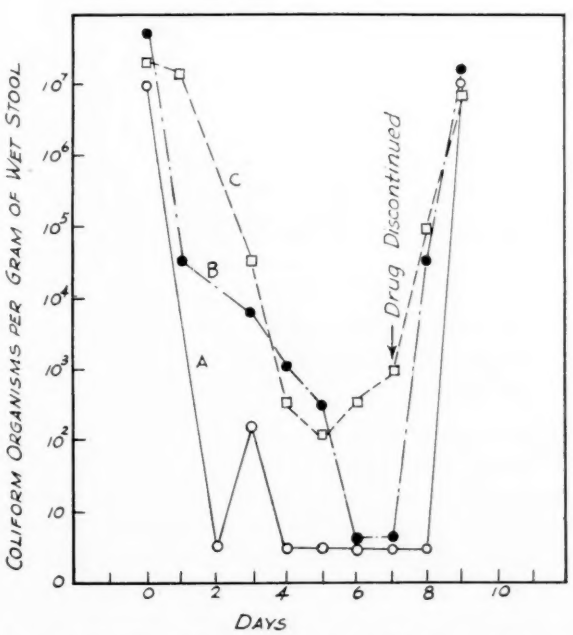

Chart 7.—The effect of spacing the oral dosage of 0.25 Gm. of phthalylsulfathiazole per kilogram of body weight daily was indicated by the change in the number of coliform bacteria in the gastrointestinal tract of man when the drug is (*A*) divided into six equal doses and given at four-hour intervals; (*B*) divided into three equal doses and given at eight-hour intervals; and (*C*) given as a single daily dose.
Compare with Charts 5 and 6.

into six equal portions reduced the coliform organisms significantly, but the reduction was not maintained. On doubling the dosage after the tenth day, the coliform count was further reduced and maintained at a low level as indicated in Chart 8. These observations further emphasize the possibility that this compound will be particularly effective therapeutically in the presence of a severe diarrhea and will permit preoperative purgation when deemed necessary to empty the large bowel.

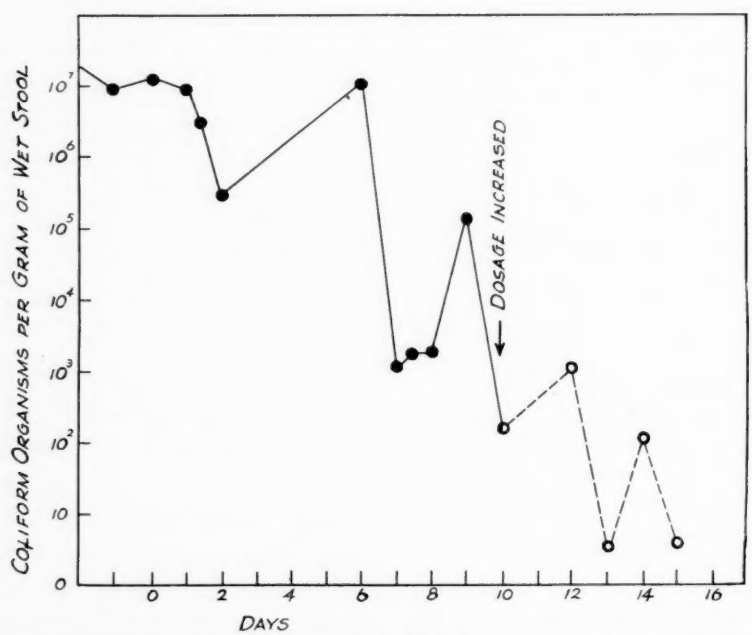

Chart 8.—Illustrating the effect of a watery diarrhea on the efficiency of phthalylsulfathiazole in altering the coliform count in the gastrointestinal tract of man. This patient received an ounce of magnesium sulfate every twelve hours for fifteen days to maintain a watery diarrhea. For the first two days 0.125 Gm. of phthalylsulfathiazole per kilogram of body weight was given by mouth at four-hour intervals as six divided doses. On the tenth day the daily dosage was increased to 0.25 Gm. per kilogram daily.

THE BACTERIOSTATIC ACTIVITY OF PHTHALYLSULFATHIAZOLE

Why should phthalylsulfathiazole have a greater local bacteriostatic activity against coliform organisms in the gastrointestinal tract than succinylsulfathiazole although the solubility of the latter exceeds that of the former compound? At least a partial answer to this question is available. If the two compounds are administered orally in equal doses and the stools are analyzed for both the free and conjugated forms of the drugs, it becomes evident, as is demonstrated by Chart 9, that both the relative and absolute concentrations of the free form of the drugs vary considerably. In the case illustrated the concentration of the free drug, which may or may not be sulfathiazole, coincident with the administration of succinylsulfathiazole attains a concentration of 250 mg. per one hundred Gm. of wet stool, while the corresponding value during the ingestion of phthalylsulfathiazole is 1,250 mg. per cent. At this dosage level, phthalylsulfathiazole is fully effective bacteriostatically against the coliform organisms while this may not be true for succinylsulfathiazole. These data would

indicate that the bacteriostatic activity parallels the concentration of the diazotizable form of the drug in the feces.

An analysis of the urinary excretion of succinylsulfathiazole and phthalylsulfathiazole on 50 successive trials of each gives the following data: (1) when succinylsulfathiazole is administered orally in a dosage of 0.25 Gm. per kilogram of body weight daily in six equal portions, the free form of the drug is excreted to the amount of 0.37 Gm. while 0.49 Gm. of the conjugated form is excreted in 24 hours, or a ratio of 1 to 1.33; (2) when phthalylsulfathiazole is likewise administered in a dosage of 0.125 Gm. per kilogram, the free form of the drug is excreted to the amount of 0.100 Gm. while 0.104 Gm. of the conjugated form is excreted, or a ratio of 1 to 1.04. In conclusion, it is obvious that relatively more of the free form of the drug is excreted when phthalylsulfathiazole is ingested, although definitely a much smaller absolute quantity of the free form of drug is excreted than when succinylsulfathiazole is administered, regardless of the fact that the concentration of the diazotizable form of drug in the feces is roughly five times as great when the phthalyl derivative is given.

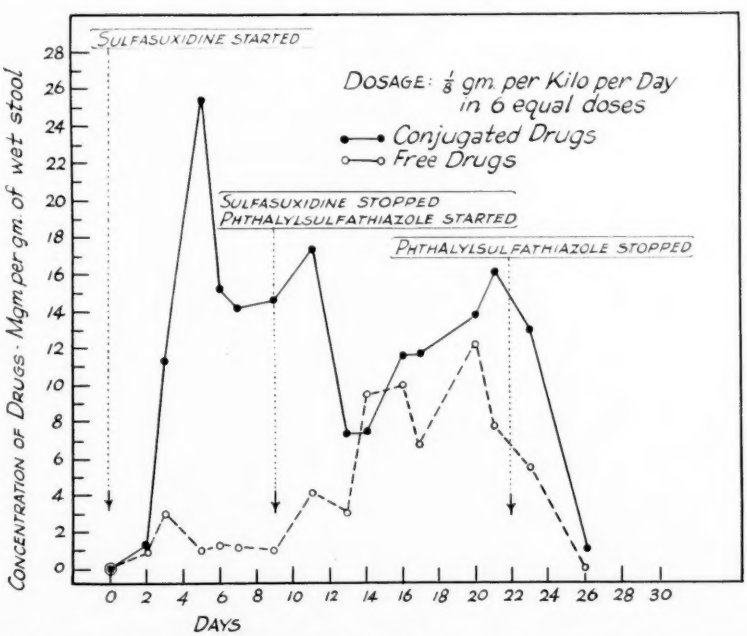
SULFASUXIDINE STARTED
DOSAGE: 1/8 gm. per Kilo per Day in 6 equal doses
Conjugated Drugs
Free Drugs
SULFASUXIDINE STOPPED
PHTHALYLSULFATHIAZOLE STARTED
PHTHALYLSULFATHIAZOLE STOPPED
CONCENTRATION OF DRUGS - Mgm per gm. of wet stool
DAYS
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28

Chart 9.—Comparison of the concentrations of succinylsulfathiazole, phthalylsulfathiazole, and sulfathiazole in the feces after the oral administration of equal quantities of succinylsulfathiazole and phthalylsulfathiazole to man.

A further analysis of the urinary excretion of the free and conjugated forms of phthalylsulfathiazole is given in Chart 10. There is little difference in the quantity of drug excreted in the urine whether a dosage of 0.125 Gm. per kilogram of body weight is ingested as illustrated in Curve *B*, or 0.065 Gm. per kilogram as shown in Curve *C*. But, it naturally follows that the percentage excreted in the second instance is roughly twice that of the first example. On the larger dosage as represented by Curve *A*, the percentage of the ingested drug excreted in the urine is least. If the urinary output of drug is determined in

a large number of cases, it is found that the quantity of drug excreted is essentially constant, which means that, within therapeutic limits, the quantity of drug absorbed from the bowel and excreted in the urine is but little affected by the ingested dose. It can, therefore, be anticipated that larger doses will not be appreciably more toxic than smaller therapeutic doses of phthalylsulfathiazole.

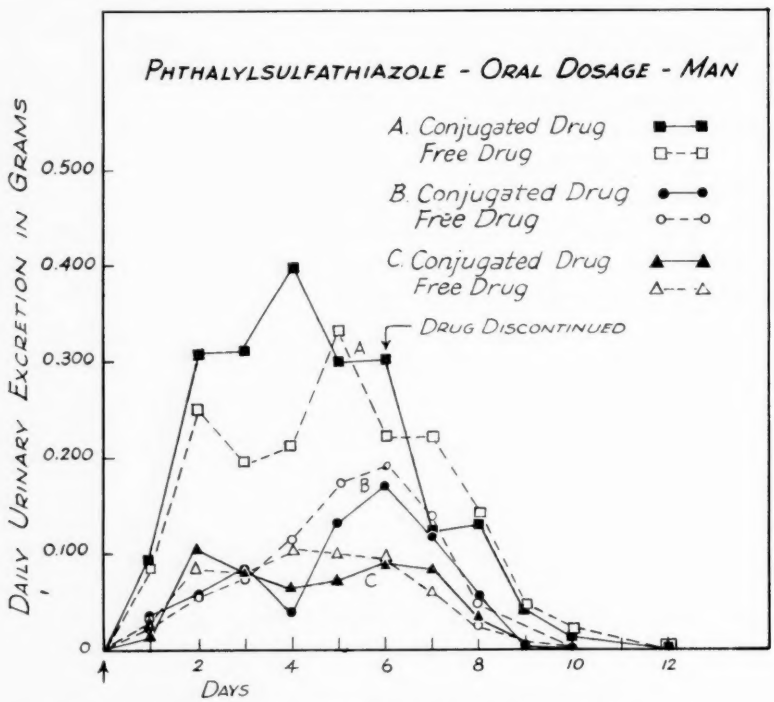

Chart 10.—Absorption and excretion of phthalylsulfathiazole by man.

A. Dosage 0.25 Gm. per kilogram per day in six divided doses at four-hour intervals by mouth; 3.35 per cent of the ingested drug recovered from the urine.

B. Dosage 0.125 Gm. per kilogram per day in six divided doses at four-hour intervals by mouth; 4.48 per cent of the ingested drug recovered from the urine.

C. Dosage 0.0625 Gm. per kilogram per day in six divided doses at four-hour intervals by mouth; 9.2 per cent of the ingested drug recovered from the urine.

An intangible mass of evidence continues to present itself that these compounds, containing free carboxyl groups in their molecules, possess antibacterial properties which are not entirely dependent upon the presence of sulfathiazole presumably resulting from hydrolysis. Although the simplest and most logical assumption would be that all the effect is contributed by sulfathiazole, it seems highly probable that the physical and chemical properties of these compounds are operative in determining their ultimate, local bacteriostatic activity by influencing the absorption and adsorption of these relatively inactive acylated compounds by the individual bacterium.

THE VALUE OF SULFATHIAZOLE AS AN INTESTINAL ANTISEPTIC

In 1942, Poth, Knotts, Lee and Inui[1] showed that sulfanilamide, sulfadiazine, and sulfathiazole had little bacteriostatic effect in the alimentary tract of the dog as demonstrated by the alteration of the coliform organisms.

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Because repeated reports have suggested that sulfathiazole and sulfadiazine have sufficient bacteriostatic activity locally in the bowel to sterilize the feces (Eisenhoff and Goldstein, 1943),[14] an additional study was made on human subjects receiving sulfathiazole in relatively large doses. Representative data are given in Chart 11. In the majority of instances, there is no significant alteration of even the coliform organisms although large doses of sulfathiazole were administered. A total of 56 twenty-four-hour collections of urine were analyzed and showed that 36 to 76 per cent, or an average of 51 per cent, of the ingested sulfathiazole was excreted in the urine. The blood levels varied from 3.4 to 7.6 mg. per cent. Assays of stools for the drug gave a concentration of 80 to 170 mg. per cent.

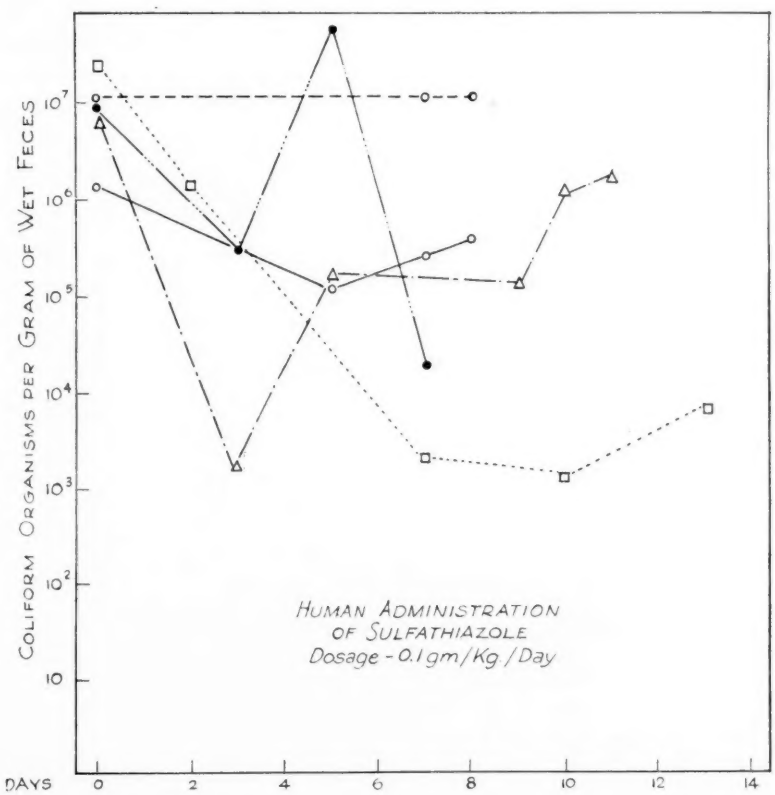

Chart 11.—A plot of the alteration of the coliform flora in the normal gastroenteric tract of man following the oral administration of full therapeutic doses of sulfathiazole. As will be noted, there may be a significant alteration in the number of coliform organisms, but this change is neither consistent nor always maintained.

In a series of several thousand stool cultures over a period of four years, we have never encountered a single instance following the administration of either succinylsulfathiazole or phthalylsulfathiazole in which the feces were sterile. It is not, however, unusual to find no coliform bacteria, and it should not be expected to find the feces free of the alpha *Streptococcus fecalis,* since the growth of this normal inhabitant of the alimentary tract is not significantly influenced by the sulfonamides. The *Shigella paradysenteriae* organisms are highly susceptible to the bacteriostatic action of the sulfonamides and show a

good therapeutic response to sulfapyridine, sulfathiazole and sulfadiazine even though it is not possible to maintain as high a concentration of these drugs in the alimentary tract as is possible when succinylsulfathiazole or phthalylsulfathiazole are administered.

THE TREATMENT OF DIARRHEAS WITH PHTHALYLSULFATHIAZOLE

Phthalylsulfathiazole has proved to be highly specific in the treatment of (Flexner) bacillary dysentery (Poth, 1943).[15] This therapeutic result could be anticipated from the experimental observations that the drug is quite effective in inhibiting the growth of coliform bacteria in the presence of a watery diarrhea (Chart 8), and that a relatively high concentration of a diazotizable derivative is maintained in the feces.

It has, furthermore, been observed that patients suffering from diarrheas, but in whom the *Shigella paradysenteriae* organisms could not be identified, frequently respond promptly following the administration of phthalylsulfathiazole. Ordinarily, the most satisfactory therapeutic regimen is to give 0.04 Gm. of the drug per kilogram of body weight every four hours for 12 doses and then 0.02 Gm. per kilogram at 4-hour intervals until diarrhea ceases or for a total

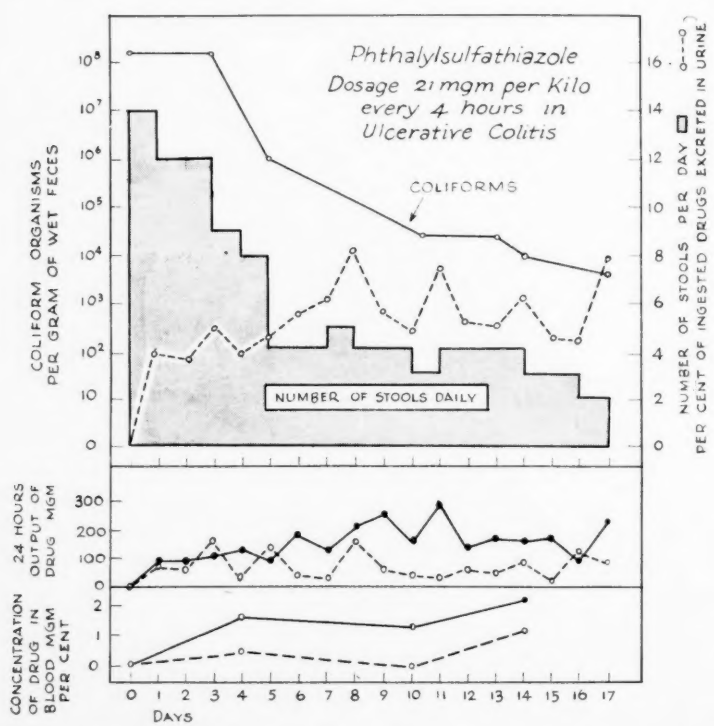
Phthalylsulfathiazole
Dosage 21 mgm per Kilo
every 4 hours in
Ulcerative Colitis
COLIFORMS
NUMBER OF STOOLS DAILY
COLIFORM ORGANISMS PER GRAM OF WET FECES
NUMBER OF STOOLS PER DAY
PER CENT OF INGESTED DRUGS EXCRETED IN URINE
24 HOURS OUTPUT OF DRUG MGM
CONCENTRATION OF DRUG IN BLOOD MGM PER CENT
DAYS

Chart 12.—The data plotted in this chart is that of D. L. B. listed in Table I. After an illness of five years' duration with practically continuous diarrhea, this patient entered the John Sealy Hospital with an abscess in right flank, which drained spontaneously and was demonstrated to be perirenal. History revealed that the abscess had been drained two years previously, and that the patient had had a daily average of 14 bloody, mucus-containing stools. Ameba and cysts could not be demonstrated in the feces. After five days' administration of phthalylsulfathiazole, the number of stools decreased to four, and they changed markedly in character. They were becoming formed and the blood and mucus practically disappeared. Subsequently the stools became odorless, formed, and of normal appearance. The patient had only one or two movements daily. The draining abscess healed. It was irrigated with azochloramide three times daily. While the chart covers 17 days, this patient received treatment for 12 weeks without evidence of toxicity and with continued improvement and weight gain. Patient taking a liberal bland diet.

of 7 days if the diarrhea is proved to be a bacillary dysentery. The feces should be negative for *Shigella paradysenteriae* on three weekly cultures following treatment if carriers are to be eliminated. Only rarely are positive stools found on follow-up cultures, and, in these instances, the individuals should be subjected to 10 days' additional therapy with 0.02 Gm. of phthalylsulfathiazole per kilogram of body weight every four hours and be observed again by culture. It is not necessarily true that the action of either succinylsulfathiazole or phthalylsulfathiazole is entirely due to sulfathiazole resulting from a simple hydrolysis of these acylated compounds. These condensation products may possess in themselves intrinsic properties leading directly or indirectly to manifested antibacterial activity.

THE TREATMENT OF ULCERATIVE COLITIS WITH PHTHALYLSULFATHIAZOLE

The treatment of ulcerative colitis will, of course, be determined by the underlying cause whenever it is possible to establish such an etiological factor. Since amebiasis may be the etiological basis of ulcerative colitis, all patients having this disease complex should receive adequate treatment with one of the amebacidal drugs even though no evidence of amebiasis can be demonstrated.

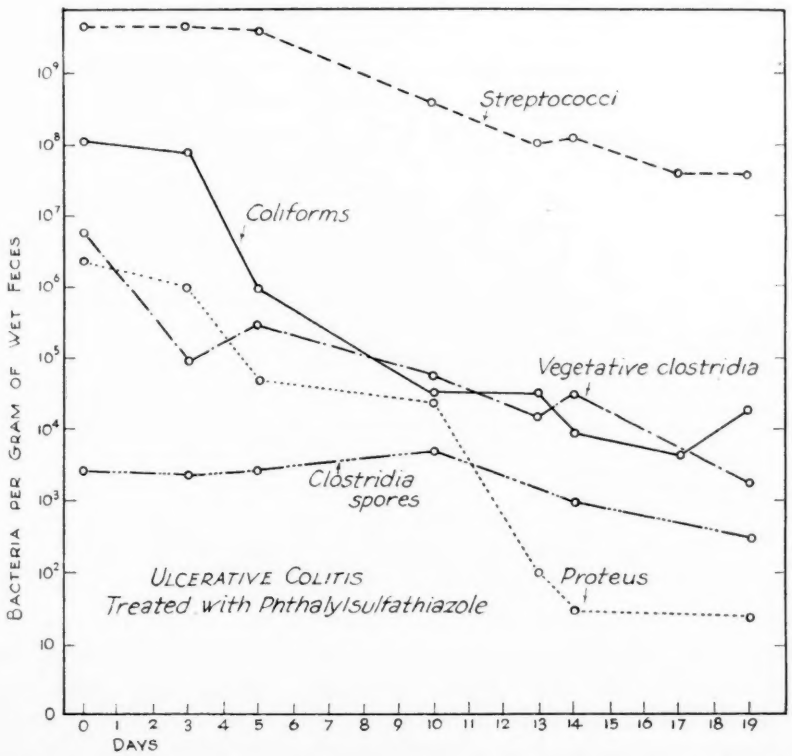

Chart 13.—A plot for the complete cultural data on D. L. B. tabulated in Table I for the initial 19 days of therapy. The rate of alteration of the bacterial flora in this patient is slower than usual because of the extensive ulcerative changes in the bowel. The entire colon has been converted to an extremely narrow tube. The streptococci are unaffected. The coliform organisms and the vegetative *Clostridia* are progressively decreasing in number. The spores of *Clostridia* have not been altered. *B. proteus* has largely disappeared. It is interesting to note that this patient had experienced considerable clinical benefit by the fifth day of therapy before there was a demonstrable change in the bacterial flora (see Chart 12). The ultimate and proper treatment will, no doubt, be colectomy, and the patient is being prepared accordingly.

Six cases of chronic ulcerative colitis have been treated with phthalylsulfathiazole. The clinical response has been satisfactory in each instance. During the initial period of four weeks, an oral dosage of 0.02 Gm. per kilogram of body weight was given every four hours. The usual early course is summarized in Charts 12 and 13.

One patient has been treated continuously for ten months with phthalylsulfathiazole without evidence of toxic drug reactions. During this period a multiple fistula in ano has healed and remained healed for three months, the bowel has regained much of its normal architecture, and the patient has had a severe upper respiratory infection without recurrence of the diarrhea which has been reduced from 10 stools containing blood, pus, and mucus to a single daily formed passage.

The number of cases treated is too small to draw any definite conclusions, but it appears that this compound is at least as effective as any of the other sulfonamides in bringing about and maintaining the patient in a remission. It deserves further study.

PHTHALYLSULFATHIAZOLE AS AN ADJUVANT IN SURGERY OF THE COLON

Succinylsulfathiazole definitely alters the postoperative course of patients upon whom operations on the colon are performed. The regimen used during the preoperative and postoperative period was outlined by us in 1942. It has been demonstrated repeatedly that such patients experience a smooth postoperative course (Allen, 1943,[16] and Behrend, 1944[21]).

Phthalylsulfathiazole may, likewise, be used. Its administration may be advantageous in those instances where the patient has a diarrhea such as is encountered, not infrequently, in the presence of malignancies of the cecum and ascending colon, as well as in those cases when it is desirable to resort to purgation to clear the bowel.

The ordinary course of preoperative preparation includes early purgation when necessary, a low residue diet or even a completely residue-free diet such as may be obtained when an amino acid-carbohydrate mixture such as "Nutramigen" is given, the administration of 0.02 Gm. of phthalylsulfathiazole per kilogram of body weight every four hours, and observation of the odor, consistency and bacterial count of the stools. As an indication of the alteration of the bacterial flora, the change in the coliform count is readily followed by the streak plate technique previously described in 1942.[17]

A small representative group of patients is presented in detail in Table I. Approximately five per cent of the ingested drug is excreted in the urine. In view of the smaller dosage of phthalylsulfathiazole as compared to that of succinylsulfathiazole only about half the absolute quantity of phthalylsulfathiazole is excreted by the kidneys.

Qualitatively the comparative effects of phthalylsulfathiazole and succinylsulfathiazole are quite similar, while quantitatively the former drug has approximately twice the antibacterial activity.

TOXICITY OF PHTHALYLSULFATHIAZOLE

There have been few toxic drug reactions seen following the oral administration of phthalylsulfathiazole. From the fact that this compound is readily

hydrolyzed in acid solution, it should follow that toxic reactions would occur more frequently than when succinylsulfathiazole is given, especially when it is observed that much higher concentrations of free drug are maintained in the colon when phthalylsulfathiazole is administered. This supposition has not been substantiated by the observed facts.

A single moderately severe toxic reaction has been observed in a 61-year-old woman with a large, redundant colon which was otherwise normal excepting for a small rectal polyp showing early malignancy. Within 24 hours after receiving 0.02 Gm. of phthalylsulfathiazole per kilogram of body weight every four hours, this patient complained of a generalized aching accompanied by headache, followed by nausea, and a chilling sensation within an additional twelve hours. A swinging type of fever developed to reach a maximum of 104.4° F. orally on the fifth day of therapy. While the patient continued to be nauseated for the entire period of drug administration, she did not vomit. The maximum concentration of the drug in the blood did not exceed 2.5 mg. per 100 cubic centimeters. The coliform organisms dropped from 10^7 to 10^2 bacteria per gram of wet stool during the five days of drug therapy. Crystalluria did not occur. No red cells appeared in the urine and there were no cytological changes in the blood. Subsequently, this patient received 0.04 Gm. of succinylsulfathiazole per kilogram of body weight every four hours, and again similar symptoms developed with nausea, headache, malaise, and a temperature elevation to 102° F. within 48 hours of administration. Obviously, this patient is sensitive to the sulfonamides.

A stoichiometric analysis of the average therapeutic dosages of sulfathiazole, succinylsulfathiazole and phthalylsulfathiazole is illustrated in Table II. The millimolar equivalent dosage of phthalylsulfathiazole is about 40 per cent that of succinylsulfathiazole and is approximately equal to that of sulfathiazole. The difference in the equivalent molecular quantities excreted in the urine is strikingly demonstrated. Fifteen times as many molecular equivalents of sulfathiazole are excreted as is the case with phthalylsulfathiazole.

Crystalluria has not been observed to follow the oral administration of phthalylsulfathiazole. The absence of crystals of drug in the urine is due to the facts that the free form of the drug is not excreted in large quantities and because the conjugated drug forms soluble salts even at a pH of 5.6.

As an increasing number of patients receive this drug, it can be expected to encounter the usual toxic manifestations of the sulfonamides, but it can be predicted that such reactions will be relatively infrequent. It must again be emphasized that any patient receiving this drug should be under constant observation.

DISCUSSION

The investigation of these various acylated sulfonamides has introduced a series of compounds which have properties such that they tend to limit their action locally to the alimentary tract because they are sparingly absorbed from the bowel. These compounds are characterized by having a free carboxyl group in the molecule and form readily soluble and highly ionized salts. It is, therefore, possible to maintain exceedingly high concentrations of these sulfonamides in the gastrointestinal tract without untoward toxic manifestations on the

TABLE I

THE DOSAGE OF PHTHALYLSULFATHIAZOLE WITH THE CONCENTRATION OF THE DRUG IN THE BLOOD AND THE FECES AND THE QUANTITY OF "FREE" AND "CONJUGATED" DRUG EXCRETED IN THE URINE

Patient and Weight in Kg.	Day	Daily Dose in Gm.	Number Doses Daily	Concentration of Drug in Blood		Daily Urinary Output in Gm.		Concentration of Drug in Stools Mg.s/Gm.		COMMENTS
				FREE	CONJUGATED	FREE	CONJUGATED	FREE	CONJUGATED	
A. B.	1	6	6	--	--	--	--			A healthy 26-year-old white female with normal gastroenteric canal used to study the effect of 0.125 Gm. phthalylsulfathiazole per kilo of body weight daily when divided into 6 doses, 3 doses, and 1 dose, taken at 4-, 8-, and 24-hour intervals, respectively. The alteration of the coliform count is presented in Chart 6. No toxic drug reaction occurred during the 49 days' administration.
45	2	6	6	--	--	0.026	0.032			
	3	6	6	0.1	0.2	0.055	0.058			
	4	6	6	0.6	0.2	0.077	0.087	4.2	17.6	
	5	6	6			0.113	0.039			
	6	6	6			0.175	0.135	2.4	7.3	
	7	6	6	0.3	0.2	0.180	0.150	9.6	7.3	
	8	6	6			0.192	0.172	10.0	11.5	
	9			0.4	0.4	0.140	0.125	6.4	11.6	
	10					0.050	0.056			
	11			0.3	0.4	0.028	0.090	4.0	2.8	Given at 4-hour intervals 4.86% of ingested drug was excreted in the urine.
	12					0.007	0.005			
	13	6	6			0.005	0.004			
	14	6	6	0.6	0.0	0.016	0.022	0.9	4.4	
	15	6	6			0.057	0.072			
	16	6	6	0.6	0.0	0.100	0.075			
	17	6	6			0.105	0.077	12.3	13.9	
	18	6	6	0.4	0.0	0.119	0.092			
	19	6	6			0.084	0.086			
	20					0.120	0.104	7.8	16.0	
	21			0.1	0.2	0.060	0.058	5.7	13.0	
	22					0.035	0.032			
	23			0.4	0.2	0.017	0.003	0.0	0.0	
	24					0.009	0.000			
	25			0.0	0.1					

	1	6	3						Drug given at 8-hour intervals; 3.7% of ingested drug was excreted in the urine.
	2	6	3						
	3	6	3						
	4	6	3			0.034	0.018		
	5	6	3	0.2	0.1	0.083	0.061		
	6	6	3			0.094	0.049	4.7	14.8
	7	6	3			0.095	0.067		
	8	6	3			0.115	0.075		
	9	6	3	0.4	0.0	0.105	0.057	9.6	35.7
	10	6	3			0.108	0.102		
	11	6	3			0.098	0.155		
	1	6	1						Drug given at 24-hour intervals; 5.1% of ingested drug was excreted in the urine.
	2	6	1	2.7	0.0			1.3	0.7
	3	6	1						
	4	6	1	0.4	3.1	0.095	0.267		
	5	6	1			0.128	0.126	20.3	17.2
	6	6	1			0.274	0.000		
	7	6	1	1.1	0.0	0.122	0.053	7.8	10.7
	8	6	1			0.147	0.036	11.6	16.4
	9					0.073	0.023		
	10			2.0	0.2				
	11								
	12							3.2	16.8
	13							0.5	0.0
S. H.	1	10	6						Woman was 52 years old. Rectal bleeding for 2 years; inoperable carcinoma of rectum; coliform count dropped from 10^6 to zero in less than 7 days; no toxic drug reactions.
39	2	5	6						
	3	5	6						
	4	5	6						
	5	5	6						
	6	5	6						
	7	5	6						
	8	5	6			0.041	0.043	14.2	34.3
	9	5	6	0.4	0.1				
	10	5	6			0.037	0.034		
	11	5	6	1.0	0.0				
	12	5	6			0.039	0.062		
	13	5	6	0.1	0.5	0.045	0.000		
	14	5	6						
	15	5	6			0.027	0.071		
	16								
	17					0.003	0.008		

TABLE I—CONT'D

Patient and Weight in Kg.	Day	Daily Dose in Gm.	Number Doses Daily	Concentration of Drug in Blood		Daily Urinary Output in Gm.		Concentration of Drug in Stools Mg.s/Gm.		COMMENTS
				FREE	CONJUGATED	FREE	CONJUGATED	FREE	CONJUGATED	
O. G.	1	12	6							57-year-old colored male; squamous carcinoma of anus and rectum; patient had no bowel movements without castor oil; coliform count reduced to zero; at operation bowel was clean and odorless; no toxic drug reactions. 5.47 per cent of ingested drug excreted in urine.
52	2	12	6	0.6	0.4	0.027	0.110			
	3	6	6	0.3	0.4					
	4	6	6			0.240	0.310			
	5	6	6	0.6	0.4	0.122	0.450			
	6	6	6			0.103	0.172			
	7	6	6	0.0	0.0	0.090	0.123			
	8	6	6			0.079	0.101	8.6	28.6	
	9	6	6	0.6	0.4	0.273	0.546			
	10	6	6			0.069	0.098			
	11	6	6	0.4	0.4	0.075	0.109			
	12	6	6			0.177	0.190	6.7	10.9	
	13	6	6	0.3	0.2	0.128	0.156			
	14	6	6			0.137	0.232			
	15	6	6			0.214	0.210			
	16	patient expired								

W. J. C.	1	9	6						63-year-old white male with an acute diverticulitis and extensive diverticulosis-dysenteric stools. Initial count 10^8; on administration of 0.125 Gm. of drug per kilo of body weight, the coliform count dropped slowly; dosage increased to 0.25 Gm. per kilo and count continued to drop to reach $10.^4$ Slow drop probably due to many diverticula out of contact with drug. Symptomatic cure of diveriticulitis; no toxic drug reaction. 3.4% of drug excreted in urine.
74	2	9	6			0.500	0.100		
	3	9	6	1.1	1.7	0.066	0.139		
	4	9	6			0.096	0.333	4.7	12.6
	5	9	6	0.5	1.3	0.085	0.300	3.4	4.7
	6	9	6			0.053	0.224	3.4	4.2
	7	9	6			0.089	0.284	2.9	4.2
	8	9	6	0.5	2.1	0.066	0.000	3.5	5.0
	9	9	6			0.126	0.178	2.5	2.0
	10	9	6			0.063	0.000	7.1	3.4
	11	18	6	1.0	0.4	0.102	0.139	4.1	5.0
	12	18	6			0.122	0.250	4.0	5.9
	13	18	6			0.330	0.337	19.6	8.3
	14	18	6	3.2	1.9	0.340	0.605	21.9	7.3
	15	18	6			0.172	0.500		
	16	18	6			0.167	0.396		
	17	18	6	0.5	5.4	0.175	0.300		
M. L.									46-year-old white male entered hospital after prolonged illness. Found to have pelvic and appendiceal abscess; developed rectovesical fistula; high urinary output due to fistula. Coliform count dropped from 10^7 to zero in 3 days; accompanying cystitis improved. Unusually high blood levels noted in isolated instances probably due to fistulas present, with absorption from granulating surfaces.
50	1	12	6						
	2	12	6	0.8	0.0	0.020	0.004		
	3	6	6	2.0	0.5	0.123	0.010	0.0	1.3
	4	6	6			0.186	0.464	2.4	5.7
	5	6	6	2.5	6.4	0.347	1.270	4.0	26.6
	6	6	6			0.476	1.476		
	7	6	6	2.7	7.0	0.737	2.513	1.8	3.8
	8	6	6			0.453	1.502	6.5	1.8
	9	6	6	2.2	6.2	1.508	0.311	2.4	2.0
	10	6	6			0.455	1.015	6.5	20.0
	11			3.2	5.3	0.897	1.178	2.2	1.5
	12			1.1	2.4	0.599	0.953		
	13			0.7	0.9	0.236	0.563		
	14					0.084	0.171	2.1	1.7
	15					0.086	0.327		
	16			0.3	0.0	0.079	0.230		
	17					0.050	0.133		
	18			0.8	0.4	0.070	0.263	0.6	0.6
	19					0.060	0.000		

TABLE I—CONT'D

Patient and Weight in Kg.	Day	Daily Dose in Gm.	Number Doses Daily	Concentration of Drug in Blood		Daily Urinary Output in Gm.		Concentration of Drug in Stools Mg.s/Gm.		COMMENTS
				FREE	CONJUGATED	FREE	CONJUGATED	FREE	CONJUGATED	
R. B.	1	3	6							White male, aged 23 years, large retroperitoneal abscess; normal gastrointestinal tract; on dosage of 0.05 Gm. per kilo of body weight, the alteration of the coliform count was irregular; but when dosage was increased to 0.10 Gm. per kilo, the count dropped to less than 1,000 in 48 hours and to zero in 5 days; no toxic drug reactions after 40 days' administration; 6.23% of ingested drug excreted in urine.
60	2	3	6	0.4	0.6	0.053	0.023	0.2	0.1	
	3	3	6	0.8	0.7	0.041	0.048			
	4	3	6	0.4	1.3	0.022	0.052	0.6	0.9	
	5	3	6	1.3	0.7	0.083	0.115			
	6	3	6	0.7	0.4	0.041	0.018			
	7	3	6	0.7	0.2	0.059	0.025			
	8	3	6	0.8	0.4	0.047	0.031			
	9	3	6			0.013	0.095			
	10	3	6	0.4	0.2	0.047	0.056	2.9	6.8	
	11	3	6			0.084	0.040			
	12	3	6	0.4	0.0	0.068	0.062	0.4	2.4	
	13	3	6			0.055	0.060			
	14	3	6	0.7	0.2	0.034	0.029			
	15	3	6			0.069	0.021	6.4	1.3	
	16	3	6	0.4	1.1	0.138	0.064	7.7	1.1	
	17	3	6			0.071	0.075	1.1	3.5	
	18	3	6	0.7	0.2	0.067	0.124	1.5	2.9	
	19	3	6			0.072	0.094	2.2	10.7	
	20	3	6	0.3	0.2	0.055	0.031			
	21	3	6			0.097	0.062	2.2	8.4	
	22	3	6	0.4	0.2	0.082	0.082			
	23	3	6			0.093	0.104	2.8	6.6	
	24	6	6	0.3	0.4	0.058	0.066			
	25	6	6			0.109	0.161	2.8	5.9	
	26	6	6	0.3	0.4	0.065	0.057			
	27	6	6			0.187	0.115	1.4	1.5	

	28	6	6	0.4	0.0	0.090	0.062		
	29	6	6			0.131	0.140	4.9	6.6
	30	6	6	0.1	0.7	0.103	0.107		
	31	6	6			0.128	0.160	3.9	2.2
	32	6	6			0.150	0.175		
	33	6	6	0.4	0.7	0.176	0.170	2.1	6.8
	34	6	6			0.218	0.281		
	35	6	6	0.6	0.7	0.160	0.193		
	36	6	6			0.168	0.259		
	37	6	6	0.4	0.2	0.105	0.116	4.0	5.1
	38	6	6			0.080	0.117		
	39	6	6	0.8	0.0	0.095	0.102		
	40	6	6			0.100	0.120		
	41	6	6	0.2	0.0	0.196	0.243	4.2	5.1
	42					0.100	0.100		
	43			0.0	0.1	0.045	0.032		
	44					0.031	0.000		
	45					0.005	0.000		
	46			0.0	0.0	0.004	0.000		
D. L. B. 52	1	6	6			0.089	0.098		
	2	6	6			0.099	0.071		
	3	6	6			0.103	0.158		
	4	6	6	1.6	0.5	0.149	0.000		
	5	6	6			0.089	0.137		
	6	6	6			0.196	0.028		
	7	6	6			0.143	0.000		
	8	6	6			0.226	0.167		
	9	6	6			0.168	0.068		
	10	6	6	1.4	0.7	0.155	0.038		
	11	6	6			0.290	0.000		
	12	6	6			0.140	0.065		
	13	6	6			0.185	0.043		
	14	6	6	2.2	0.9	0.163	0.088		
	15	6	6			0.168	0.000		
	16	6	6			0.092	0.098		
	17	6	6			0.240	0.091		

30-year-old male; history of chronic ulcerative colitis of long standing; diarrhea for past 5 years; received sulfanylguanidine about 2 years ago and suffered severe toxic reaction without alteration of diarrhea. For alteration of flora and diarrhea upon administration of phthalylsulfathiazole see Charts 12 and 13. No toxic reaction to phthalylsulfathiazole. 5.4% of ingested drug excreted in the urine.

TABLE II

THE COMPARATIVE MILLIMOLAR DOSAGE AND URINARY EXCRETION OF SULFATHIAZOLE, SUCCINYLSULFATHIAZOLE, AND PHTHALYLSULFATHIAZOLE BY MAN

AVERAGE THERAPEUTIC DOSE	EQUIVALENT DOSAGE IN MILLIMOLES	AVERAGE URINARY EXCRETION EXPRESSED IN MILLI-EQUIVALENTS PER KILO OF BODY WEIGHT
Sulfathiazole 0.10 Gm./kilo/day (Molecular weight 255)	0.392	0.235[1]
Succinylsulfathiazole 0.25 Gm./kilo/day (Molecular weight 355)	0.704	0.035[2]
Phthalylsulfathiazole 0.125 Gm./kilo/day (Molecular weight 403)	0.310	0.0155[2]

[1]Assuming 60 per cent of the ingested drug to be excreted in the urine. (Barlow and Climenko, 1941.)[18]

[2]Assuming 5 per cent of the ingested drug to be excreted in the urine.

part of the host. The local bacteriostatic action may arise from several sources of which the following immediately present themselves: (1) the intrinsic action of the drug per se and its anion conditioned by its physical and chemical properties to influence the local concentration of the compound within and upon the individual microorganisms dependent upon the specific absorption and adsorption prevailing; (2) the simple hydrolysis of the conjugated drugs to yield the so-called "free" sulfonamides; (3) the formation of compounds in a nascent or excited state by the action of the microorganisms upon the absorbed or adsorbed acylated derivatives to yield substances possessing apparent specific activity merely because the thermodynamic environment is such as to favor the formation of an active compound in a relatively high concentration in the immediate vicinity of or within the cell of the susceptible microorganisms; and, (4) the influence of split products other than the sulfonamide radical; i.e., succinic acid from succinylsulfathiazole. Succinate is of importance in the substrate concerned in certain oxidation-reduction chains involved in respiratory enzyme systems. Such a process may alter the normal metabolism of the cell or even tend to eliminate or prevent the formation of substances which inhibit the more specific competition of the sulfonamides to fix an hypothetical substance required as a normal metabolite by the organisms.

It is not clear that the free or diazotizable form of compound present in the feces following the administration of either phthalylsulfathiazole or succinylsulfathiazole is sufathiazole or even some other single compound, because the quantity of diazotizable substance excreted in the urine following phthalylsulfathiazole administration is less than one-half the quantity following the administration of succinylsulfathiazole although in the former instance, a diazotizable substance is present in the feces in five times the concentration as is the case during succinylsulfathiazole administration.

It is logical, therefore, to anticipate that these drugs may exhibit individual bacteriostatic properties dissimilar to each other as well as to sulfathiazole. Such dissimilarities may permit one to hope that various of the condensation products might possess individual properties giving these compounds a degree of specificity against different species and strains of bacteria.

Regardless of any theoretical consideration of mechanisms as to action, the fact remains that these drugs induce profound alterations in the bacterial flora of the alimentary tract reducing certain organisms to a very small percentage of the initial population while other organisms appear completely resistant. Because of the fact that bowel contents are not rendered sterile, but rather that the bacterial flora is merely simplified, the value of inducing such an alteration in the preoperative preparation of the colon has been questioned. Meleny and his co-workers[6] have shown that mixtures of certain bacteria are roughly 15 times as virulent as their pure strains. It has been demonstrated by Poth, Knotts, Lee, and Inui[1] and confirmed by Saltzstein[19] that succinylsulfathiazole can so modify the contents of the large bowel of the dog that gross drainage of the descending colon into the peritoneal cavity does not result in fatal peritonitis. Sarnoff and Poth[20] have shown that dogs can be protected against necrosis of segments of the ileum following ligation of the venous return by adequately treating the animals with succinylsulfathiazole prior to the ligation.

If the bacteriostatic activity of phthalylsulfathiazole as regards coliform organisms can be taken as an index of the relative antibacterial activities of phthalylsulfathiazole and succinylsulfathiazole for all bacteria, then one can predict that the former possesses roughly twice the activity of the latter compound. This relationship will not, however, be true if either of these compounds or their degradation products have any specificity for any species, type or strain of organisms.

CONCLUSIONS

Phthalylsulfathiazole, a condensation product of sulfathiazole and phthalic anhydride, is an antibacterial agent of considerable interest and of therapeutic possibilities where activity restricted to the alimentary tract is desired. Approximately five per cent of the orally administered therapeutic dose is excreted in the urine. Ordinarily, the concentration of the drug in the blood does not exceed 1.5 mg. per 100 cubic centimeters. As compared to their respective bacteriostatic activities, when measured by their ability to suppress the coliform organisms, phthalylsulfathiazole possesses roughly twice the activity of succinylsulfathiazole. In the absence of diarrhea and ulcerated lesions in the bowel, a single daily dose of phthalylsulfathiazole will effectively lower the coliform organisms in the feces.

The vegetative forms of the *Clostridia* are greatly reduced following the oral administration of phthalylsulfathiazole, and stools are rendered essentially odorless without ordinarily producing a diarrhea. The drug is shown, likewise, to be an effective bacteriostatic agent locally in the bowel as is indicated by the alteration of the coliform bacteria in the presence of a watery diarrhea.

An extensive study of absorption and excretion has shown that an average of 5 per cent of the oral, therapeutic dose of phthalylsulfathiazole is excreted in the urine. Analyses of stools reveal that the content of phthalylsulfathiazole and a "free" diazotizable degradation product chemically similar to sulfathiazole varies between wide limits and that this "free" compound may maintain a concentration of 1,250 mg. per cent.

Preliminary trials of phthalylsulfathiazole in nonspecific diarrheas, bacillary dysentery, chronic ulcerative colitis, and for the preoperative preparation of the large bowel are presented. The drug appears particularly well tolerated by patients having ulcerative colitis and is quite effective in inducing and maintaining prolonged remissions of the disease. Severe toxic manifestations have not been encountered in ulcerative colitis patients even though the therapy has continued for several months. Phthalylsulfathiazole can be maintained in high concentration in the diseased alimentary tract of man with low concentrations of the drug in the blood.

Some of the possible mechanisms through which the acylated sulfonamides act are discussed, and it is proposed that the action of succinylsulfathiazole and phthalylsulfathiazole may not be due either wholly or in part to the formation of sulfathiazole by simple hydrolysis.

As indicated by the alteration of the coliform flora in the bowel of man, phthalylsulfathiazole, *in half the dosage,* is as effective as succinylsulfathiazole.

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THE ACUTE TOXICITY OF COMMERCIAL PENICILLIN

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THE large volume of clinical data collected during the relatively short time that penicillin has been used presents extremely strong evidence of the innocuous nature of this drug. The clinical evidence has been obtained on a variety of diseases through the efforts of the Office of Scientific Research and Development in carefully organized clinical studies. Such an accomplishment in normal times would have required years of effort by individual investigators. From the original studies of Chain, et al.[1] and Abraham, et al.,[2] it has been apparent that even with relatively crude preparations there was little to indicate any contraindication to its use in man. Later studies by Hobby, et al.,[3] Herrell, et al.,[4, 5] Blake and Craige,[6] and Rammelkamp and Keefer,[7] have all substantially confirmed the fact that at least so far as penicillin sodium is concerned, little, if any, toxicity can be ascribed to this therapeutic agent. It is true that recently Lyons[8] has reported an occasional, at present unexplained, sensitization but such a "toxic" effect, if it may be so described, is apparently associated with individual idiosyncrasy rather than with penicillin itself. As pointed out by Lyons, "No significantly harmful effects have been observed."

In testing commercial penicillin for "toxicity" a method has been used which involves the intravenous injection of mice with 100,000 units per kilogram of body weight. Five mice are injected and the product is considered satisfactory if none dies within a forty-eight-hour period of observation. In carrying out routine tests of this nature it soon became obvious that such a test does not measure the "toxicity" of penicillin but rather constitutes a safety test which is capable of eliminating only those products seriously contaminated with substances toxic for mice. In the assay of over 300 lots of penicillin sodium produced for clinical use by fourteen different manufacturers none has failed to pass the safety test. Similarly, commercially produced penicillin calcium and penicillin ammonium were found to be satisfactory when tested by the above method. A commercial preparation of penicillin magnesium, however, failed to pass the safety test, but in this instance the toxicity, as described later in this report, was shown to be due to the cation rather than to penicillin itself.

In order to determine the acute toxicity of commercial penicillin sodium, the material remaining from each lot after assay, which includes tests for sterility, potency, moisture content, safety, and pyrogens, was injected intravenously into mice in increasing dosages. In some instances it was not possible to establish the "toxic level" of units with individual lots because of lack of material. However, it was possible to establish a "pattern" of the acute toxicity of each manufacturer's product by studying several lots, either indi-

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vidually or pooled. At least three mice were injected at each level of units tested and repeat tests were made at the dose found to be lethal to the extent of the material available for testing.

TABLE I

ACUTE TOXICITY OF PENICILLIN SODIUM IN MICE

MANUFACTURER	NO. OF LOTS TESTED	TOXIC DOSE UNITS/20-GRAM MOUSE*	APPROXIMATE RANGE UNITS/MG.
A	12	3,500- 4,500	100-275
B	6	5,000- 8,000	46-120
C	11	7,000- 9,000	130-320
D	7	8,000-18,000	100-346
E	13	10,000-16,000	140-750
F	8	10,000-16,000	140-330
G	7	12,600-14,000	300-400
H	11†	15,000-18,000	200-500
	5†	8,500- 9,500	134-160
I	15	13,000-21,000	250-540
J	12	25,000-32,000	300-500

*All mice observed for 48 hours.

†Recent lots of this manufacturer's product have shown a lower potency and correspondingly higher toxicity.

In Table I are given the number of units of penicillin sodium which consistently caused death of 20-gram mice when injected intravenously. There is a marked difference in the number of units found to be invariably lethal for mice with the various manufacturers' products. The product of manufacturer A in a study of 12 lots consistently killed 20-gram mice in doses of from 3,500 to 4,500 units, while the product of J consistently killed mice at 25,000- to 32,000-unit dosages. It is of interest that an occasional lot of manufacturer J's product failed to kill mice when injected in 30,000- to 35,000-unit doses in 20-gram mice, while manufacturer A's product has never been found to be innocuous in doses greater than 4,500 units per 20-gram mouse. The toxicity of the penicillin sodium produced by manufacturers B to I, inclusive, falls between 5,000 and 21,000 units. In studying a number of lots of penicillin from different manufacturers over a period constituting the first three months of commercial production, it has been apparent that in some cases at least there has been a general decrease in the toxicity of the products produced. As an example, manufacturer E, who produced penicillin which was toxic at levels of from 7,000 to 8,000 units per 20-gram mouse in early production is now producing penicillin which is toxic for mice at from 14,000 to 16,000 units per 20-gram mouse. There has been a corresponding change in the potency. In early production the potency was from 200 to 300 units per milligram, while recent lots range from 700 to 1,000 units per milligram. Similarly, eleven lots of penicillin produced by manufacturer H, which assayed 200 to 500 units per milligram, were found to be toxic at 15,000 to 18,000 units per 20-gram mouse, while a more recent group of five lots assaying 134 to 160 units per milligram proved to be toxic in doses of 8,500 to 9,500 units per 20-gram mouse. This manufacturer recently reduced the purity of his preparation by changing his extraction method in order to increase his yield of penicillin.

In studies of six different salts of penicillin it has been shown[9] that the toxicity is due mainly to the cation used in the preparation of these salts rather

than to the penicillin itself. Some evidence of this nature will be given later in this report. In the case of penicillin sodium the cation does not appear to be responsible, particularly with those products found toxic at low unitage. (See Table II.) In a previous study[10] it was demonstrated that there are substances elaborated in the production of penicillin by the autolysis of the penicillium mycelia which are both pyrogenic and toxic for mice. It would appear, therefore, that the toxicity at lower unit levels of some manufacturers' products is associated with their methods of extraction of penicillin from the crude metabolic solution. It is of interest, however, that with manufacturer J's product, which was found to have an L_D-50 in mice (intravenous injection) of 28,000 units, the cation is responsible for the toxicity. This manufacturer's product had an average potency of 408 units per milligram and thus the L_D-50 in milligrams of penicillin sodium was 68.6. Since chemical assay showed the presence of 11.7 per cent Na^+, there were 8.0 mg. Na^+ present at the L_D-50. The L_D-50 of Na^+ of sodium acetate was found to be 7.8 milligrams. It is apparent, therefore, that the toxicity in this relatively atoxic product is primarily due to the cation.

COMPARISON OF THE ACUTE TOXICITY OF A CALCIUM AND A SODIUM SALT OF PENICILLIN

Through the courtesy of manufacturer I it has been possible to study the acute toxicity of a calcium and a sodium salt of penicillin, both prepared commercially from a single master lot of material. The potency of the calcium salt was found to be 310 units per milligram with a moisture content of 2.17 per cent, while that of the sodium salt was 332 units per milligram, with a moisture content of 2.0 per cent. Chemical assay of these salts showed the presence of 8.16 per cent Ca^{++} and 9.97 per cent Na^+. To determine the acute toxicity of these two salts at least three mice were injected intravenously in the tail veins with increasing amounts of each salt until a level was reached at which the product was found to be no longer innocuous. The calcium salt was lethal for 20-gram mice in doses of 3,500 units, while a dose of 21,000 units of the sodium salt was required to bring about the same result. From a clinical standpoint this marked difference in toxicity of the sodium and calcium salts may have little significance since on a weight basis 3,500 units of the calcium salt would be equivalent to a single injection of over ten million units in a 60-kilogram man.

On the basis of potency it is obvious that the greater toxicity of the calcium salt cannot be associated with penicillin as such. During the preparation of the salts they had equal opportunity of picking up, from the master lot, substances toxic for mice. To what degree this occurred should be dependent upon their individual combining power with such substances. It would appear, however, that the demonstrated greater toxicity of the calcium salt is not the result of its selective combining power with toxic organic material in the master lot of penicillin since its purity (310 units per milligram) is not much less than the purity of the sodium salt (323 units per milligram).

In attempting to find the cause of the greater toxicity of the penicillin calcium, an L_D-50 was determined in mice for both the penicillin calcium and for calcium acetate. The L_D-50 for the calcium salt of penicillin was found to

be 3,700 units per 20-gram mouse. Since chemical assay showed the penicillin to contain 8.16 per cent Ca^{++} and its potency was found to be 310 units per milligram, the L_D-50 in terms of Ca^{++} is computed to be 0.97 milligrams. The L_D-50 of Ca^{++} of calcium acetate was found to be 1.03 mg. per 20-gram mouse. Therefore, the toxicity of the penicillin calcium is primarily due to the cation used in its preparation, and little, if any, toxicity can be ascribed to organic contaminants or to penicillin itself. Similar tests were made with penicillin sodium and with sodium acetate. In this case the sodium salt had a lethal dose of 21,000 units, a potency as noted above of 323 units per milligram, and it contained 9.97 per cent Na^+. The number of milligrams Na^+ at the lethal dose was therefore 6.31. Since the lethal dose of Na^+ as sodium acetate was found to be 6.7 mg., it appears that the cation was largely responsible for the toxicity of this sample of penicillin sodium.

TABLE II

THE TOXICITY OF THREE SALTS OF PENICILLIN VS. THE TOXICITY OF THE CORRESPONDING ACETATES

MICE	UNITS PER 20 GM.	MG. PER 20 GM.	MORTALITY RATIO*	MICE	MG. PER 20 GM.	EQUIV. UNITS PENICILLIN PER 20 GM.	MORTALITY RATIO*
	Penicillin Sodium				*Sodium Acetate*		
6	3,500	2.97	0/6	6	6.30	7,500	0/6
6	4,000	3.36	4/6	6	6.70	7,900	2/6
6	4,500	3.78	4/6	6	7,60	9,000	3/6
	Penicillin Magnesium				*Magnesium Acetate*		
6	600	0.276	0/6	9	0.267	580	0/9
6	700	0.322	2/6	7	0.312	670	2/7
6	800	0.367	5/6	6	0.367	798	3/6
	Penicillin Ammonium				*Ammonium Acetate*		
6	4,500	1.23	0/6	6	1.50	5,500	0/6
3	4,700	1.32	2/3	6	1.80	6,600	2/6
6	5,500	1.50	2/6	6	2.10	7,700	4/6
6	6,000	1.64	6/6				

*48-hour observations. Mortality ratio: Ratio of number of mice dying to total number injected.

COMPARISON OF THE ACUTE TOXICITIES OF THE SODIUM, MAGNESIUM, AND AMMONIUM SALTS OF PENICILLIN WITH SODIUM, MAGNESIUM, AND AMMONIUM ACETATES

In view of the results obtained above with a relatively atoxic lot of penicillin sodium, a comparison was made of a more toxic lot of penicillin sodium with sodium acetate. Since both penicillin magnesium and penicillin ammonium have been recommended for clinical trial, similar comparisons were made also with these two preparations. All three penicillin samples tested were commercial preparations. The tests were carried out in a manner similar to that recorded above and the results are given in Table II, where it will be noted that with the sample of penicillin sodium tested, 4,000 units caused death in mice. Since this product contained 84 mg. of Na^+ per 100,000 units, there were 3.36 mg. of Na^+ at the 4,000-unit dose. When comparison is made with sodium acetate it will be noted that the lethal dose of Na^+ of sodium acetate is 6.7 milligrams. Therefore, the toxicity of this sample of penicillin sodium is not due to any great extent to the cation, but rather to either penicillin itself or, more

likely, to organic contaminants resulting from the process of extraction, all of which can be removed by proper extraction methods. The results obtained with this sample of penicillin sodium are in marked contrast to those obtained on the sample of penicillin sodium referred to above which had a lethal dose of 21,000 units. It is of interest that the penicillin sodium referred to in Table II produces marked local reactions in the doses used clinically.

The penicillin magnesium preparation is extremely toxic in comparison to penicillin sodium or penicillin calcium. In the routine safety test, which consists of the injection of five 20-gram mice with 2,000 units each, all animals died within a few seconds, and it was not until the product had been diluted approximately 1:3 (to 600 units) that the preparation became innocuous. It should be pointed out, however, that the penicillin magnesium was a low-potency material (70 units per milligram). The L_D-50 of this sample of penicillin magnesium was found to be 710 units per 20-gram mouse. Since the potency of this product was 70 units per milligram and it contained 3.22 per cent Mg^{++}, the number of milligrams of Mg^{++} at the L_D-50 is therefore 0.325 milligrams. The L_D-50 dose of Mg^{++} of magnesium acetate was found to be 0.38 milligrams. In the case of this preparation of penicillin magnesium, the toxicity of the product is primarily associated with the cation. Another sample of penicillin magnesium, prepared in these laboratories and having a potency of 1,028 units per milligram, was found to have an L_D-50 for mice of 7,600 units. However, again with this high potency preparation, the cation appears to be responsible for the toxicity. Since the L_D-50 was 7,600 units and the potency of the material 1,028 units per milligram, the L_D-50 in milligrams of penicillin is 7.3 milligrams. Chemical assay showed the presence of 4.23 per cent of the cation. Therefore, at the L_D-50 there were present 0.31 mg. of Mg^{++}, which corresponds quite closely to the milligrams of Mg^{++} present (0.38) at the L_D-50 of magnesium acetate. The results obtained with both high and low potency preparations of pencillin magnesium tend to question the clinical use of such material particularly for intrathecal injections.

The lethal dose of penicillin ammonium is approximately 4,700 units (Table II). At this lethal dose there were found present by chemical analysis 1.32 mg. of NH_4^+. By comparison, 1.8 mg. NH_4^+ were found to be present at the lethal dose of ammonium acetate. In the case of penicillin ammonium, the cation, although not primarily responsible for the toxicity of this preparation, probably contributed substantially to the toxic effect. The commercial preparation of penicillin ammonium was relatively insoluble in water, except in very low concentrations. When this preparation was dissolved in water at concentrations of 4,000 units per cubic centimeter a precipitate resulted which could not be dissolved even with the application of heat. The precipitate, however, contained little or no potency, since assay of the supernatant fluid indicated that all of the penicillin was in solution. In performing the tests given in Table II the penicillin ammonium was dissolved in water, the solution centrifuged, and the precipitate discarded before injection of the mice. However, the penicillin solution, on standing for a relatively short time, reprecipitated and it was not possible at any time during the study completely to clarify it. It is quite possible that this precipitate contributed to the toxicity of this

penicillin preparation. In any case it would appear unlikely that commercial penicillin ammonium in its present state of purity is satisfactory for clinical use in man.

SUMMARY AND CONCLUSIONS

A study of over 300 lots of penicillin sodium produced by fourteen manufacturers showed all to pass the mouse safety test.

There is a wide variation in the acute toxicity of different manufacturers' products. Some products are lethal for mice in concentrations of 3,500 to 5,000 units while one manufacturer is producing penicillin sodium lethal only at doses of 25,000 to 32,000 units.

A comparison of commercial samples of penicillin calcium and penicillin sodium made from a single master lot showed the calcium salt to be by far the more toxic preparation. The greater toxicity of the calcium over the sodium salt of penicillin should not discourage its clinical use in man. The toxicity of the penicillin calcium is primarily due to the cation.

The toxicity of high potency samples of penicillin sodium and high or low potency samples of penicillin magnesium is primarily due to the cation used in producing these preparations. Similarly, the cation contributes substantially to the toxicity of penicillin ammonium.

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ACTINOMYCOSIS

Report of a Case With Miliary Chest Lesions

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BOLLINGER, in 1877, first described the disease of cattle known as "lumpy jaw." He attributed the cause to *Actinomyces bovis*. In 1878 Esrael reported a case in man. The following year Ponfick showed the essential similarity of the organisms causing these conditions in man and cattle.

Actinomycosis enjoys a wide distribution, even though infrequent in occurrence. According to Cope,[4] about 65 deaths result yearly from this disease in Great Britain. Auster[1] found the condition more prevalent in grain-producing areas of the United States. Davis,[5] however, reports an even distribution of actinomycosis between rural and urban populations.

The actinomyces constitute a very commonly encountered group of organisms. Zinsser and Bayne-Jones[11] found it in soils and occasionally in normal mouths. Microscopically, the organism has a branching filamentous structure with a peculiar predisposition to form into clumps or colonies. The central portion of these clumps appears as a mass of debris with radiating filaments and club forms around the periphery. This structure gives rise to the term "sulfur granules." Generally these sulfur granules can be seen grossly and are considered diagnostic. Actinomycosis really connotes a group of diseases, just as the actinomyces belong to a group of organisms rather than a single pure strain. Some of these organisms have aerobic qualities, while others are anaerobic. Most of them take the Gram stain. A few are acid-fast. The fact that several types of organisms occur has led to considerable controversy. Likewise, numerous names have been given to different forms of the disease caused by these various strains. The following quotation from Foulerton[7] indicates an early appreciation of the confusion: "Whilst in this country and the United States the term streptotrichosis has been adopted generally, continental pathologists as a rule still favour the older designation for these infections, actinomycosis. And, writers on general medicine have not always appreciated the fact that these two terms are absolutely synonymous; so that one sometimes finds even in recent books reference made to the streptotrichoses as if they were in a class of disease in some sort different from the actinomycosis."

Recently the controversy regarding the classification and nomenclature of the *Actinomyces* has been greatly clarified by the work of Waksman and Henrici.[10] According to their classification, the common type, as seen in lumpy jaw, has anaerobic characteristics and receives the name of actinomycosis. The aerobic form with the tendency to reproduce by fragmentation of the mycelium is designated "Nocardia." They also mention its frequent acid-fast features.

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The diagnosis rests on finding the organism in the lesions. Usually one sees the typical "sulfur granules." When difficulty in finding sulfur granules arises, however, stains may be employed to demonstrate the individual organisms. Pathologically, the lesions produced are characterized by a large amount of fibrosis, some necrosis, and the presence of a purulent exudate containing polymorphonuclear leucocytes. The picture somewhat resembles tuberculosis and other granulomatous lesions, except for the more acute type of exudate. Sometimes giant cells appear.

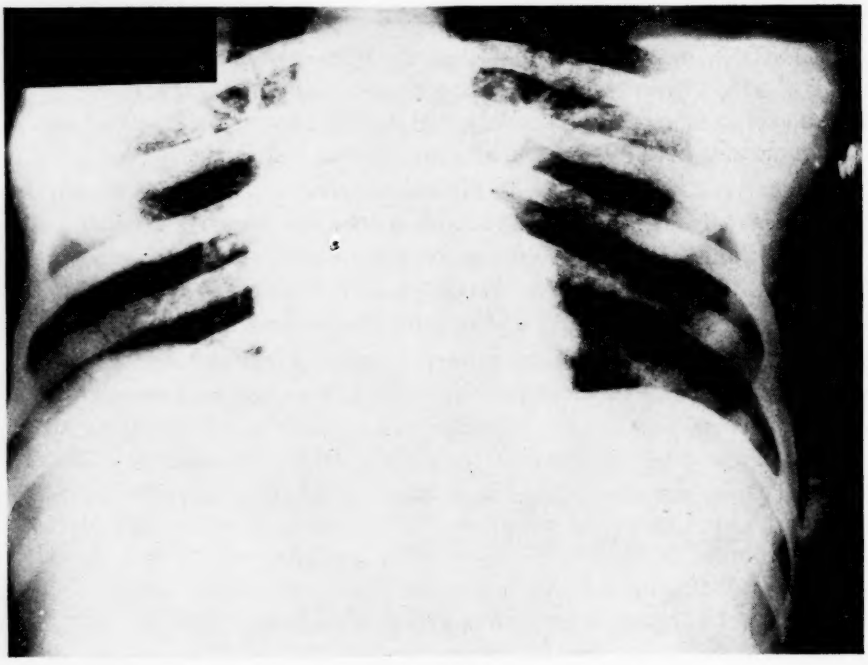

Fig. 1.—Photograph of roentgenogram of the chest and upper abdomen, showing miliary actinomycosis.

Cecil[3] groups cases of actinomycosis according to their clinical manifestations in the following manner: (a) The most common type involves the head and neck. It comprises 50 per cent of the reported cases. The mouth serves as the source of infection. (b) The next most common type comprising 20 per cent to 30 per cent of all cases occurs in the abdomen. This type most frequently follows an appendectomy or other similar procedure. Characteristically, numerous sinuses develop. (c) The third, or pulmonary, type comprises approximately 15 per cent of the total. In addition to these three more common types, other rare forms involving the skin or mucous membranes and the generalized form occur. Most writers on the subject mention the possibility of generalized spread.

In 1934 Biggart[2] reported the case of a woman who developed a lesion on the thigh. The infection spread rapidly and death occurred within three months. The autopsy findings included generalized actinomycosis lesions of the lungs, liver, spleen, and kidneys. The case herewith presented has similar features.

The disease commonly runs a chronic course lasting from months to years. There have been a large number of complete recoveries reported. In general, however, the prognosis is not too favorable. According to Davis,[5] the cervicofacial type offers the best prognosis. Wangensteen[9] emphasizes the poor prognosis of actinomycosis involving the chest or abdomen.

In the treatment the single most important factor is good surgical care. Wangensteen[9] emphasizes the importance of this. According to him, as the organism is an anaerobic one, the removal of dead tissue and debris is necessary in order to do away with media for the growth of the organism and to aerate the tissues. Numerous drugs have been used. Dobson[6] found sulfanilamide helpful in some cases. The action of the drug in actinomycosis appears to be on the secondary invaders rather than the primary infection. Joyce[8] recommends the use of thymol in conjunction with radical surgery. A number of authors, including Dobson, recommended radiation with the thought that the resulting fibrosis tends to wall off the infection. The iodides have been used for some years. Best modern opinion regarding treatment seems to be the use of radical surgery in connection with thymol and radiation.

CASE REPORT

The soldier, a Negro male aged 23 years, entered the hospital June 9, 1943, complaining of a painful swelling in the left axilla of eight days' duration. He recalled no injury. Previous to admission he received several days' treatment with hot packs and sulfathiazole; the condition grew worse.

The salient features of the physical examination on admission consisted of several firm, tender, nonfluctuating, enlarged axillary glands. One group felt conglomerated. The temperature registered 102°, the pulse 88, and respiration 20 per minute. Examination of the blood showed 19,000 white blood cells with a secondary anemia.

Four days after admission, incision of the left axilla produced 30 c.c. of thick, white pus. No sulfur granules were visible grossly and the culture grew no organisms. Early treatment, consisting of incision and drainage with chemotherapy, resulted in no improvement. The high temperature continued. The blood culture was reported negative. Ten days later the axilla was again incised and a second incision on the anterior portion of the chest was made. This drained several pockets and infected the tracts. Through-and-through drainage was established, and a piece of tissue was removed for biopsy. Upon re-examination of the pus and from the biopsy, the diagnosis of actinomycosis was established. Patient then received iodides both orally and by vein, thymol, transfusions, and plasma. No improvement was observed, however. The temperature remained very high, seldom below 101°. The white blood cells numbered 35,000. The number of immature forms increased as the condition progressed. Four weeks after admission an enlargement resembling a left inguinal adenitis appeared. This immediately suggested a metastatic abscess. Upon incision a few days later, pus disgorged. In spite of excellent nursing care the patient developed two decubitus ulcers on the back. Roentgen therapy produced no beneficial results. One month after admission, the presence of pulmonary findings was noted. Chest films at this time revealed multiple deposits distributed evenly through the parenchyma of both lungs, although a film eleven days previously was reported negative. Frequent nosebleeds became troublesome. Death occurred seven weeks after admission, following several irrational days.

Laboratory diagnosis was made on the basis of smears and tissue sections. The smears showed the frequent presence of an acid-fast organism. This organism was a long, slender rod with a fairly marked tendency to produce poorly developed buds. Except for the budding tendency, the large number of organisms present, and the predominance of cells with a segmented nucleus, the picture resembled that commonly seen with the tubercle bacillus. Sections of the actinomycotic tissue showed a ground work of relatively avascular gran-

ulation tissue. Fibroblasts, reticulocytes, and endothelial cells predominated. Scattered through the tissue appeared numerous small areas of necrosis surrounded by many polymorphonuclear neutrophiles. No organisms took the ordinary hematoxylin-eosin stains. Acid-fast stains showed the same organisms seen in the smears. Mallory's stain for actinomycosis was not helpful, except for one slide which had a small, but typical colony of so-called "sulfur granules."

A guinea pig inoculated with material from the sinus tract died the same day as the patient. It showed lesions quite similar to those seen in the patient. The organisms demonstrated in the guinea pig tissues were identical to the organisms seen in the human tissue. No growth was obtained on Sabouraud's or other media commonly used for fungi.

AUTOPSY FINDINGS

External Examination.—This is the body of a fairly well-developed, thin, young adult Negro male estimated to be 5′7″ tall and weighing approximately 130 pounds. There is an ulcerated area in the left inguinal region with an opening of a sinus tract. There is a decubitus ulceration in the left costovertebral angle and also over the sacrum. There is a 9 cm. recent surgical incision just lateral to the border of the pectoralis muscle on the left. This leads into a sinus tract in the soft tissues over the chest. There is a 2 cm. incision in the belly of the muscle to provide through-and-through drainage.

Peritoneum.—The liver border is 4 cm. below the right costal margin. The spleen is at the left costal margin. The peritoneal cavity contains about 50 c.c. of blood-tinged serous fluid. There are no adhesions in the peritoneal cavity.

Pleural Spaces.—On removing the sternum the thoracic duct is seen to be swollen and filled with a mass of inflammatory tissue. Pleural spaces contain no excess free fluid. There are no adhesions in either pleural space.

Heart.—Essentially normal.

Lungs.—The right lung weighs 915 grams. There is some atelectasis posteriorly. The hilar lymph nodes are enlarged. Bronchi are filled with blood-tinged frothy material. The cut surface presents a congested appearance with considerable edema and multiple miliary lesions. There is considerable consolidation of the tissue.

The left lung weighs 820 grams. The atelectasis is least marked. The cut surface is essentially similar to the right. In some areas there is beginning cavitation. The miliary lesions are uniform in size, the largest measures approximately 3 mm. in diameter.

Liver.—Weight of the liver is 2,310 grams. The capsular surface is studded with miliary lesions. The cut surface is also completely taken up with these lesions. All of them are small and the largest measures approximately 1 mm. in diameter.

Spleen.—Weight of the spleen is 480 grams. The capsular and cut surfaces are studded with many miliary lesions, the largest measures approximately 4 mm.

Pancreas.—Normal.

Kidneys.—The right kidney weighs 195 grams. The left kidney weighs 240 grams. There are fairly numerous miliary lesions in both kidneys. The largest measures about 3 mm. The lesions are similar to those seen in other organs except there are not so many of them in the kidney as in other tissues.

Adrenals.—The medullary portion is hard to differentiate from the cortex.

Miscellaneous.—There is some enlargement of the mediastinal and mesenteric lymph nodes. There is a mass of enlarged lymph nodes in the region of the celiac axis. These lymph nodes on cut section present a matted necrotic appearance. Several areas in the nodes contain purulent material. Dissection of the left axilla shows the sinus to be multilocular and to extend beneath the scapula and into the subclavicular region. It does not, however, penetrate the pleura.

MICROSCOPIC SECTIONS

Heart.—Normal.

Lungs.—Scattered through the lung tissue, rather uniformly, are small areas of necrosis. These areas have some mononuclear reaction surrounding them; in general, however, the cellular response is minimal. An occasional multinucleated giant cell of the Langhans type is seen. Miliary lesions are scattered throughout all the lung sections.

Liver.—Scattered through the liver sections are miliary lesions which are similar to those described in the lung. The cellular response is again minimal.

Spleen.—The spleen contains a large number of miliary lesions similar to those seen in the lung. Many of the lesions in the spleen are larger than those seen in the lung or liver. In other respects they are similar.

Pancreas.—There are, in isolated areas, occasional miliary lesions similar to those seen in other organs.

Kidney.—Scattered through the tissue there are fairly numerous miliary lesions similar to those seen in other organs. It is noted that these lesions are frequently in the areas of the arcuate arteries or in the pyramids.

Adrenals.—Adrenals are normal, except for the presence of fairly numerous miliary lesions similar to those seen in other organs.

Lymph Nodes.—Sections through the lymph node show a much more passive necrosis than that seen in other areas. The follicular structure is almost entirely replaced. Edema is a fairly prominent feature. Again the paucity of a cellular response is noted.

Miscellaneous.—Acid-fast stains were done on many of these tissues. These sections show the presence of an acid-fast organism in all of the areas where miliary lesions are encountered. These organisms are for the most part long slender nodes with a tendency to appear in small groups or clumps and a marked tendency to produce branching forms. The large number of organisms encountered is an interesting feature. Another characteristic is the tendency for the organisms to be distributed widely. Mallory's stain for actinomycosis was not particularly helpful.

CONCLUSIONS

1. A case of generalized actinomycosis showing interesting miliary lung lesions is reported, with autopsy findings.

2. The first clinical manifestation appeared in the axillary glands, suggesting the infection entered an abrasion of the hand or arm.

3. The final pathologic picture resembled in many respects the end result in miliary tuberculosis. The finding of inflammatory tissue in the thoracic duct suggests this origin for the hematogenous spread.

4. All modern methods of treatment were employed, including adequate surgery, chemotherapy, roentgen therapy, thymol, iodides, and supportive treatment. None of these appeared appreciably to affect the progress of the disease

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LOCAL EOSINOPHILIA IN MALIGNANT NEOPLASMS

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IT IS the purpose of this paper to review briefly the question of local eosinophilia in cases of malignant tumor, especially as observed in carcinoma of the cervix uteri.

Eosinophile infiltration has been observed in many instances in the stromal tissues of a variety of neoplasms. I have noted that the phenomenon is most striking and most frequent in cases of epidermoid carcinoma of the cervix. In the typical case, which shows this special reaction, the neoplasm itself is not remarkable in appearance and there is no very impressive histological peculiarity which sets such tumors apart, except for the marked local eosinophilia. I am not aware, either, of any unusual features of clinical appearance or history in these cases prior to biopsy.

The inflammatory reaction in the stroma of carcinoma of the cervix is somewhat variable, although, in most instances there is a rather marked infiltration of lymphocytes, plasma cells, and moderate numbers of large mononuclear cells. Polymorphonuclear neutrophiles occur with somewhat less frequency. Eosinophiles are present in the vast majority of cases, but usually are few in number, and widely scattered. They are probably not much more numerous than might be expected in many chronic inflammatory processes. In a small percentage of cases these cells appear to be absent. In another small group of carcinomas of the cervix, there is an infiltration of eosinophiles which is definitely greater than that seen in the great majority and which, in a few cases, exceeds the number of all other inflammatory cells present.

The characteristic lesion of the type under consideration reveals a moderately undifferentiated epidermoid carcinoma, in the stroma of which there is a dense infiltration of typical eosinophiles. Occasionally the tumor stroma is entirely obscured by the large number of these cells which lie closely packed together.

From January 1, 1938, to August 4, 1943, there have been approximately 361 biopsy fragments of carcinoma of the cervix received in this laboratory, of which 317, from 309 patients, were available for study in the preparation of this paper. Only eight of the 317 cases studied were adenocarcinomas and none of the eight showed the change in question. Each slide has been examined and an estimate made as to the frequency with which eosinophiles appear among the inflammatory cells in the stroma. As noted in a previous paragraph, most of the cases reveal small numbers of eosinophiles. An effort was made to pick out only those in which eosinophilia was definitely greater than could be seen in the majority of the slides studied. It was decided to attempt further division of the cases, which showed eosinophilia greater than usual, into four grades of eo-

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sinophile density, designated one plus to four plus. This division was based upon an estimate of numbers of cell types in all parts of the tumor stroma. It should be mentioned at this point that the distribution of inflammatory cells is often variable and there are sometimes rather marked differences in the type of exudate from one part of a section to another part of the same section.

I have chosen to base the one plus to four plus degrees of eosinophilic infiltration upon the following estimates of numbers—i.e., one plus equals any increase in eosinophiles above that seen in the majority of cases, to about 25 per cent of the total inflammatory cells present; two plus equals an estimated 25 to 50 per cent eosinophilia; three plus equals 50 to 75 per cent eosinophilia; four plus equals 75 per cent or greater infiltration of eosinophiles. I wish to emphasize that these figures do not represent actual counts of cells, but are estimates based upon a survey of all parts of a single slide in each case.

Of the 317 biopsies studied, a total of 26 slides, from 24 patients, revealed a local eosinophilia which definitely exceeded that commonly seen in the other 291 biopsies. The subdivision into groups revealed the following: one plus, 17 cases; two plus, 2 cases; three plus, 3 cases; four plus, 4 cases. Thus it can be seen that 7.7 per cent of the cases in this series show what I believe to be a significant local eosinophilia; and in 7 (2.2 per cent) of these, it appears that eosinophiles make up more than half of all inflammatory cells present. There is almost invariably a heavy inflammatory cell infiltration in those cases of marked local eosinophilia, and there were, therefore, very large numbers of these cells present.

From January 1, 1938, to September 21, 1939, there were 104 biopsies of carcinoma of the cervix studied from a total of 97 patients. Two of this group were adenocarcinomas. Eight biopsies revealed significant eosinophilia in a total of six patients. Three biopsies from one patient over a period of about six weeks, each revealed a marked eosinophilic reaction.

Complete follow-up information on cases from January 1, 1938, to September 21, 1939, was available on 53 of the patients of the original group of 97. Only one of this group of 53 is alive at present. This case failed to show the inflammatory reaction under consideration. Final follow-up data were available on all 6 of the patients who showed noteworthy eosinophilia in the biopsy tissue. Analysis of the 53 cases reveals that the average duration of life after biopsy of the 6 notable cases was approximately seventeen months, whereas the remaining 47 lived an average of approximately fourteen months. This is perhaps suggestive but not considered a definitely significant difference in such a small series. There appears to be no important difference in longevity between various degrees of local eosinophilia in this series.

It is obvious that there are many opportunities for error and misinterpretation of findings in a group of this type. There is, of course, a large personal factor in the judgment of what does and does not constitute a degree of local eosinophilia in the one plus group of cases, which is definitely greater than that observed in the majority of slides. Another possibility for error lies in the smallness of the series of patients on whom up-to-date data could be obtained. It is possible and even probable that those patients who cannot be traced are more likely to be alive and are lost to sight because of change of address, name,

etc. It is probable, then, that some of the ninety-seven patients on whom follow-up data was not available, are still living although all but one of the fifty-three patients traced successfully to date are dead. In determining longevity, based upon length of life, from biopsy to death there is undoubtedly considerable error because of the wide differences that exist in various patients as regards their response to early symptoms of disease and the time which was allowed to elapse between onset of symptoms and appearance of the patient in a clinic for biopsy.

It appears that the type and extent of the inflammatory reaction seen in connection with carcinoma of the cervix, probably does not depend upon any obvious features of invasion, character of tumor, or degree of superficial necrosis except where the biopsy fragments include necrotic debris and exudate from surface ulceration. In the superficial tissues, of course, where extensive ulceration and secondary infection are marked, there is usually an exudate, the cellular portion of which is composed chiefly of polymorphonuclear leucocytes. This is usually quite different from the type of inflammation which occurs deep in the stroma and which may represent resistance to tumor invasion. In a very few instances there is little or no inflammation in the stroma of the tumor. Most of this latter group tend to be rather well-differentiated tumors and often reveal an extremely dense, relatively acellular type of stroma.

It has been felt by many observers that inflammation in neoplastic processes might represent resistance of the host. Ewing[2] notes that inflammation often meets the invasion of tumor tissue and that this is a significant defensive process.

It is interesting to speculate as to the causes of appearance of inflammatory cells in general and eosinophiles in particular as a response to tumor invasion. Dead and dying cells and many bacteria are chemotactic for inflammatory cells and it is not unlikely that degenerations of tumor tissue alone might account for exudate in and about the tumor in many cases. As soon as ulceration and secondary infection occur there is ample explanation for infiltration of inflammatory cells of various types. As regards the attraction of eosinophiles, Ingraham and Wartman[4] observed that eosinophiles are strongly attracted by certain bacteria and other substances in a manner comparable to the response of neutrophiles to the same chemotactic substances. They suggest that it is possible that eosinophiles may be attracted to a site of inflammation in the same manner as polymorphonuclear neutrophiles.

Apropos of tissue breakdown and foreign protein reaction, it has been shown by Biggart[1] that foreign protein injected in muscle results in a polymorphonuclear leucocyte response about the site within twenty-four hours, but at the end of about eight days the reaction is predominately eosinophilic. It appears, however, that in animals already showing a general eosinophilia, the injection of proteins causes a local reaction which may be predominately eosinophilic from the first. Sterile necrosis from turpentine injection gives similar results.

The question as to the origin of eosinophiles has come up from time to time in the past and there has been some difference of opinion as to their histogenesis. It is believed by some that tissue eosinophiles may be developed from lymphocytes, plasma cells and perhaps other cell types in the tissues and that many

eosinophiles seen in various conditions are formed locally and do not necessarily arrive from the blood. Biggart[1] feels that there is no distinguishing feature between eosinophiles found in tissues and those of blood. He concludes that the tissue eosinophile "is an emigrated eosinophile leucocyte" and that "local eosinophilia occurs at the expense of the circulating eosinophiles." He lists many conditions in which he has observed eosinophilia. His list includes among others, inflamed appendices; chronic gastric ulcer; epithelioma of hand, head, and tongue; carcinoma of the thyroid, and a variety of carcinomas of the gastrointestinal tract. He makes the interesting observation that every neoplasm of the gastrointestinal tract examined by him showed a local eosinophilia, regardless of the presence or absence of secondary inflammatory changes.

The suggestion is made by Biggart that asceptic necrosis of an animal's own tissues will set free chemotatic factors, probably protein in nature, for eosinophiles; and that eosinophilia in tumors, whose protein metabolism is especially active, and in which there is constant aseptic destruction of normal and malignant tissue cells, probably is of this nature.

A number of reports have appeared which suggest some immediate value to be derived from the observation of unusual eosinophilia in connection with neoplastic diseases, quite apart from the more academic questions as to the why and wherefore of the phenomenon. Goforth and Snoke[3] regard the accumulation of many eosinophiles locally as evidence of body resistance to neoplasia. They report a case of carcinoma of the cervix with marked local eosinophilia in which the patient exhibited prolonged resistance to the progress of the tumor process, and they state that in instances where local eosinophilia has been a feature, the patient has shown greater resistance to the process, has responded better to treatment and lived longer than when eosinophilia was absent. It is their opinion that the presence of the eosinophile is a good omen in carcinoma of the cervix.

Schoch[6] examined 417 cases of carcinoma of the cervix by biopsy and noted eosinophilia in 10 per cent. Three hundred fifty-nine of the patients in this series died in five years. Of this number 9 per cent showed eosinophilia. In the cases which remained cured for five years (58 cases) eosinophilia was noted in 40 per cent. The proportion of cures in the total number of cases (417) was 13.2 per cent; in the 40 cases with eosinophilia, cures were 45 per cent. All cases received radiotherapy. These are interesting observations although there appears to be some discrepancy in the figures.

Pavlovsky and Widakowich[5] observe that eosinophiles seem to have a protective action against cancer invasion and that in malignant disease with marked eosinophilia, the course is more favorable.

The causes of the marked local infiltration of eosinophiles in some cases of malignant neoplasm are, as yet, obscure. None of the usual explanations for eosinophilia seem entirely adequate. It is possible that special conditions of necrosis with liberation of protein degeneration products, or unusual bacterial infection of ulcerated lesions, or perhaps a strong allergic susceptibility of an individual to either protein products of degenerated tissues or bacteria, may account for this phenomenon. It is unlikely, in my opinion, that there is any special tumor tissue which is specifically chemotactic for eosinophiles. It seems

much more reasonable to assume that the reaction is due to some change, probably degenerative, in the tumor or in the tissue of the host or in both. This may not be different from an allergic reaction of the host to a protein antigen.

SUMMARY

What is thought to be a significant degree of local eosinophilia was observed in 7.7 per cent of a series of 309 cases of epidermoid carcinoma of the cervix.

There is some reason to believe that the presence of eosinophiles in abundance in the stroma of malignant tumors is of good prognostic import (other factors being equal), and probably represents a better than usual resistance to the advance of the neoplasm.

It is hoped that future long-term observations with follow-up cases will confirm the value of this phenomenon as a favorable indication in prognosis.

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SUBACUTE BACTERIAL ENDOCARDITIS CONFINED TO A PULMONIC VALVE WITH MALFORMED LEAFLETS

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ENDOCARDITIS confined to the pulmonic valve is of infrequent occurrence, single cases having been reported by De Candole,[1] Lehman,[2] Leader,[3] Grayzel,[4] and Cockayne and Wilton.[5] In all but one of these, Leader's Case, the endocarditis was of the acute variety in keeping with Allen's[6] observation that there seems to be a generally greater tendency for the virulent rather than the relatively avirulent organisms to involve the right side of the heart. Libman[7] reports that 26.8 per cent of cases of acute endocarditis involve the right side of the heart, and that only 1 per cent of the subacute cases affect that side. Denman[8] and Thayer[9] reporting separately on subacute endocarditis, place the incidence of cases in which the pulmonic valve alone is affected at 2 per cent and 1 per cent, respectively. Clawson and Bell[10] found no cases confined to the pulmonic valve in their series of eighty cases.

Aside from its unusual location as a subacute lesion, the following case of endocarditis presents findings of more than passing interest, foremost of which is a pulmonic valve in which two of the leaflets are rudimentary. Another interesting feature is the fact that the right side of the heart showed no evidence of hypertrophy or dilatation, despite the marked pulmonary valve insufficiency caused by the defective leaflets and the superimposed inflammatory destruction.

CASE REPORT

The patient, F. B., was a white male, aged 47 years, a meat cutter by occupation.

The family history contained nothing of significance. The past history states that he had had tonsillitis and joint pains as a young man. He had always been weak, asthmatic, and troubled with dyspnea.

Present Illness.—About six months before admission to the hospital he developed a dry cough with occasional hemoptysis. One week before admission he became ill with chills, fever, malaise, aching in muscles and joints, pleuritic pains, nausea, vomiting, and loss of appetite. He had lost twenty pounds in the last six months. After hospitalization the patient had intermittent febrile episodes, the temperature ranging from 99° F. to 105° F. He complained of cough, and pain in the chest and abdomen.

Physical Examination.—The patient was pale, emaciated, ill appearing, with no cyanosis, dyspnea, or edema. There were dullness on percussion, harsh breath sounds, râles, and a pleural friction rub in the left lower chest. The peripheral vessels were not sclerosed, the pulse rate was 120; rhythm regular. The apex impulse was in the fifth interspace 8 cm. from the midsternal line. The pulmonic second sound was accentuated with reduplication. There was a three plus rather harsh systolic murmur over the pulmonic area. There was a soft high-pitched diastolic murmur along the left sternal border. The liver and spleen were not palpable.

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Laboratory.—Chest roentgenograms showed increased density in the bases of both lungs. The heart shadow was normal. Electrocardiograms were normal. Blood serology was negative. Four blood cultures were positive for *Streptococcus viridans*. Complete blood count showed red cells, 3,900,000; white cells, 12,700; hemoglobin, 85 per cent; polymorphonuclear cells, 88 per cent; sedimentation rate, 24 mm. in one hour.

Clinical Diagnosis.—Subacute bacterial endocarditis involving the pulmonic valve.

Course.—Patient failed steadily; the diastolic murmur at the left sternal border increased in intensity. Death occurred twenty weeks after admission.

Fig. 1.—Photograph of pulmonic valve and wall of right ventricle showing the two rudimentary leaflets on the right, and the large pendulous vegetation just left of center.

Note: The malformed leaflets are so shallow as to be scracely discernible in the photograph.

Autopsy.—Inspection revealed an emaciated white male. There was no edema. The sclerae and skin were normal. The heart weighed 325 grams. There was no dilatation or hypertrophy and the myocardium was of normal color and consistency. The mitral, aortic and tricuspid valves were normal. The pulmonic valve (Fig. 1) consisted of an anterior cusp with a complete but thickened leaflet, and remnants of the right and left cusps, made up of two narrow, slightly elevated, white bands, representing the proximal portion of the right and left semilunar leaflets. The right cusp remnant was thickened and rough near its commissure with the anterior cusp. A polypoid greenish-gray vegetation measuring 3 by 1.5 cm. was attached to the commissure, between the anterior and the right cusp. This was suspended on a narrow stalk, and its dependent portion was thick and globular. There were several small, slightly elevated vegetations seen on the intima of the pulmonary artery just above the valve, some reddish and others greenish-gray in color. The coronary arteries were not thickened and the aorta was smooth.

The lungs showed patchy red areas in the lower lobes, and a few droplets of white pus were expressed from the cut surfaces. The liver and spleen were grossly normal. Small pinhead-sized abscesses were seen on the cut surfaces of the kidneys.

Microscopic.—Serial sections taken from the left cusp, Fig. 2, showed almost complete absence of the leaflet, except for a very small budlike projection of fibrosa. This was rounded and smooth, covered by endothelium, and an intact elastic lumen could be demonstrated under the endothelium with Weigert's elastic tissue stain.

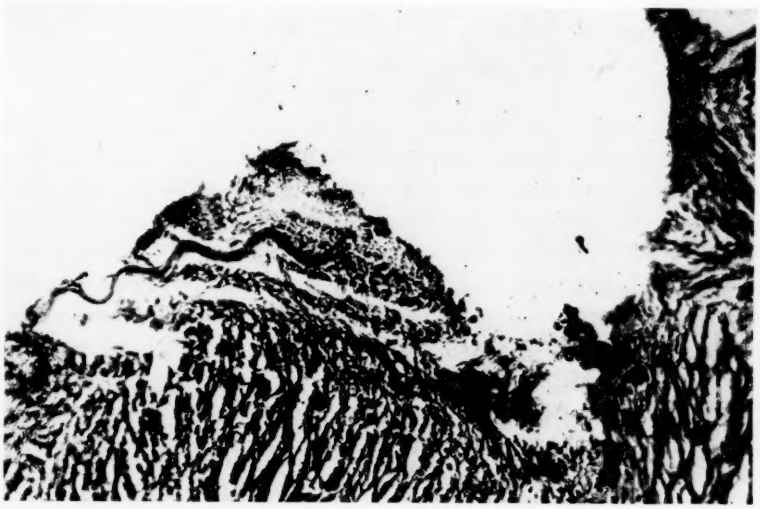

Fig. 2.—Microphotograph of section through the left pulmonary cusp. The subendothelial elastic lamina is clearly defined in the budlike leaflet.
Weigert's elastic tissue stain ×300.

Fig. 3.—Photomicrograph of section through the right pulmonary cusp. There are many neutrophils and phagocytes within the fibrosa with loss of structure. Part of a small vegetation with its group of polymorphonuclears is seen at the lower edge.
Hematoxylin and eosin ×300.

Sections taken from the right cusp, Fig. 3, showed a hyalinized vegetation attached to the lumen side of the leaflet, within which many polymorphonuclear neutrophiles were to be seen. The valve spongiosum (not seen in the illustration) showed the presence of many newly

formed capillaries and young fibroblasts. No evidence of rheumatic involvement could be demonstrated in serial sections of the myocardium and leaflets. Bronchopneumonia and infected emboli could be demonstrated in the sections of lung.

DISCUSSION

Since no rheumatic stigmata were found either grossly, or in serial microsections of the valves and myocardium, it is quite obvious that the "locus minoris resistentiae" for the *Streptococcus viridans* was the defective pulmonary valve leaflets. To determine whether these defects were congenital or acquired requires careful analysis of the gross and microscopic findings. The fact that no other anomalies were present in the heart is of little significance. Abbott,[11] in her tabulation of one thousand cases of congenital heart disease, reported 43 cases of congenital bicuspid valves unaccompanied by other defects and 125 cases in which other anomalies were noted. We may conclude from Abbott's findings that the presence of other congenital anomalies makes it more likely that a given lesion is congenital, while their absence does not exclude the probability of a congenital etiology. De Vries[12] considered that all cases in which the pulmonary valve is affected (by endocarditis) are due to maldevelopment. Koletsky,[13] referring to bicuspid pulmonary valves, states that they are all of congenital origin and that as far as is known, no acquired deformity of this type has ever been observed.

The microscopic findings strongly indicate, if they do not prove, that the defective leaflets are of congenital origin.

Fig. 2 is a photomicrograph of a section through the budlike prominence representing the rudimentary left leaflet. The fibrosa is covered by unbroken endothelium and the elastic laminae are intact. These findings, particularly the latter, are not what would be expected in inflammatory destruction followed by repair. It is true that the intact endothelium means little by itself, since regeneration of endothelium is a common accompaniment of intimal repair; however, it is hardly conceivable that extensive inflammatory destruction would leave an intact subendothelial elastic in the remaining portion of the leaflet, nor is it likely that repair would so closely simulate the normal relationship between fibrosa elastica and endothelium.

Assuming for the moment that the cusp remnants are congenital anomalies, we are offering the following hypothesis taken in part, from Simonds[14] to explain their maldevelopment. In the developmental division of the common arterial trunk into the aorta and pulmonary artery, two pairs of swelling develop on the inner surface of the vessel. These four swellings are anlagen of the semilunar cusps; later the lateral pair become divided in their longitudinal axis when the common trunk forms the aorta and pulmonary artery; this results in two groups of three pads, one group in each vessel. The semilunar leaflets are formed when these pads are indented, or hollowed out from above. Incomplete hollowing or shallow indentation of the pads from above would prduce defects in which the leaflet would be relatively shallow or deep, according to the point at which the process was arrested.

Definite proof that a given lesion of a heart valve is congenital is possible in some instances. For bicuspid aorta and pulmonary valves, particularly for the former, well-defined gross and microscopic criteria have been developed to differ-

entiate between congenital and acquired lesions. The work of Bishop and Trubek[15] and later by Koletsky[16] provides definite gross and microscopic differentiating points, the main one being the relationship in the raphe of the elastic media with the annulus fibrosa. In congenital cases the media is superficial to the connective tissue of the annulus fibrosa, which is the reverse of the normal relationship, in which the annulus is superficial to the elastica. Obviously, such criteria is of no help in determining the nature of the defective leaflets found in our case since we are not concerned here with a raphe; however, the microscopic findings, the intact subendothelial elastica, and the absence of signs of inflammation in the left cusp seem adequate to establish the defect as congenital, while that in the right cusp, with all the evidence here of inflammatory destruction, may be purely inflammatory or due to combined maldevelopment and inflammation.

The *a priori* effect of a pulmonary valve insufficiency of over 60 per cent, i.e., almost total loss of two leaflets, would be hypertrophy, dilatation, or both, of the right auricle and ventricle. As noted in the case report and autopsy, such effects were not present. The electrocardiogram was normal, and the heart was of normal size and shape without evidence of hypertrophy or dilatation in any of its chambers. This apparent incongruity demands some explanation. Fig. 1 shows the defective pulmonic valve and the large polypoid vegetation on its stalk. It was noted at autopsy that the globular dependent portion of the vegetation occupied the space within the valve ring usually closed by the right and left leaflet. It seems probable that the vegetation had some compensatory effect on the insufficiency by inhibiting the back flow of blood into the right ventricle during diastole; in other words the mobile, rounded vegetation functioned somewhat as a ball valve, moving upward with the blood stream in systole and dropping back into the ring in diastole, thus serving to diminish the effects of the incompetent leaflets. Another point to consider is the probability that the valve was competent before the onset of the endocarditis. This seems likely since many congenital defects are silent clinically; e.g., in Koletsky's report of nine cases of congenital bicuspid pulmonary valves, he states that they were silent and were found to be of little, if any, clinical significance. The valves functioned competently in all instances, with no regurgitation or stenosis at the valvular orifice.

SUMMARY

This case of subacute bacterial endocarditis was confined to a pulmonic valve in which there was almost complete absence of two of the leaflets, the underlying etiology being congenital maldevelopment. Despite the marked insufficiency, there were no demonstrable changes in the myocardium of the right side of the heart, a fact partially explained by the compensatory action of a large pedunculated vegetation which functioned as a ball valve.

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A NOTE ON A POSSIBLE ALLERGIC FACTOR IN ALTITUDE SICKNESS

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EVIDENCE has been accumulated in Mexico City (altitude 7,325 feet) which indicates that allergic reactions are more common at that altitude than at lower altitudes. From this I have deduced *Corrolary No. 1:* Mountain sickness, symptoms of which are the same as those of allergic reactions, may be explained on an allergic basis, and *Corrolary No. 2:* The higher the altitude, the more allergy will be encountered. Therefore, everyone may be potentially allergic and may develop some type of allergy if he stays at a high enough altitude long enough. The importance and practical application of this is that aviators flying at high altitudes may increase their efficiency and avoid unpleasant reactions by taking precautions used in allergic cases.

These points are to be supported by case histories and data on 500 cases 15 years of age and under, arranged in alphabetical order and taken from my files without selection of any kind. The cases were about equally distributed as to sex. There were 350 American or European and 150 Mexican patients.

In support of the theory that allergic reactions are more common at higher than at lower altitudes, I shall present data regarding hives and urticaria, and regarding gastrointestinal symptoms.

As a basis for comparison with my data, I have chosen the experiences of Vaughan[1] summarized in his statement: "I believe that we shall eventually find ourselves within striking distance of the actual facts when we accept the statement that approximately 10 per cent of the population suffers from frank allergy, major allergy, while an additional 40 or 50 per cent have experienced or will experience some minor evanescent allergic manifestations, not sufficiently pronounced to require medical consultation."

I. Hives, Urticarial Rashes and Eczema.—

Out of 500 individuals, 167 had either severe hives, urticarial rashes or eczema, there being 25 cases of the latter.

The diagnosis of hives was made on the basis of (1) appearance, (2) location and the ruling out of insect bites, (3) the proved relation to taking certain foods three or more times and improvement on omitting them three or more times, (4) the occurrence often coincident with gastrointestinal upsets. The diagnosis of eczema was made on the basis of characteristic appearance and location usually in antecubital and popliteal spaces.

II. Gastrointestinal Disturbances: Diarrhea, Nausea and Vomiting.—

In this group many things must be considered in differential diagnosis. These cases are not so obviously allergic on first inspection as hives and eczema. However, the following points are considered in ruling out other conditions:

1. Low blood count.
2. Negative stool cultures.

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3. Relation to certain foods: giving certain foods causes symptoms, and removing these foods causes improvement.

4. Relation to hives and urticarial rashes, both gastrointestinal disturbances and skin conditions often occurring simultaneously.

Of the 500 cases, 100 had diarrhea, vomiting accompanying this in 48 individuals. The most common offenders were eggs, milk, orange juice, and chocolate in that order. Symptoms in most of these cases became acute within three weeks of arrival in Mexico City. Later, symptoms are still present but in a more chronic form such as anorexia, failure to gain weight, fatigue, irritability. These symptoms clear up on finding and removing the offending foods from the diet. These reactions are definitely not due to some specific abnormality of food or water in Mexico City, as many children develop hives, diarrhea and vomiting traceable to food products imported from the United States such as cod liver oil, canned evaporated or powdered milk. These cases clear up when the particular food is omitted from the diet or when the patient goes to a lower altitude where he may take that food.

CASES

B. B., female, born April, 1940. Sept. 6, 1941. Sudden urticaria on back, right arm, fingers and legs, following breakfast of Pablum, orange juice, milk and soft boiled egg. Eggs were suspected, but four foods were omitted from the diet: orange juice, tomato juice, chocolate and eggs. This restriction plus enema and laxative resulted in prompt subsidence of urticaria. Oct. 24 to Jan. 3, 1942, the patient was in Los Angeles, Calif., and there ate oranges and eggs without any difficulty. On Mar. 26, 1942, the child had had severe itching urticarial rash all over body for five days, getting worse. She had been taking eggs and orange juice since her return Jan. 3, 1942. Treatment was enema, laxative, and omission of orange juice and eggs. The rash began to subside the next day. Apr. 14, 1942. Her mother reported that eggs without orange juice caused a rash, and that orange juice given alone caused no trouble.

B. B., male, born June, 1939. Aged 4 years. He has mild eczema in Mexico City and not in Indiana. He can take fresh cow's milk and evaporated milk in Indiana, but can take only small amounts of condensed milk here.

A. A. B., born June, 1937, and seen first Aug. 10, 1940, soon after arrival in Mexico City, because of vomiting. Physical examination gave no explanation for vomiting and temperature was normal. Urine was +++ for acetone. On Sept. 30, 1940, the patient had another attack of vomiting which lasted only one day. On Oct. 15, 1940, she was vomiting again, and was advised to omit eggs from the diet. By Dec. 21, 1942, the child's mother had repeatedly observed that trouble was related to ingestion of egg, and that the child has a decided reaction even to small amounts. She had never had any such trouble before coming to Mexico City.

P. C., male, born May 24, 1935. Oct. 16, 1942. Patient was first seen shortly after arrival in Mexico City because of vomiting, and was found to be acidotic (acetone ++). As vomiting continued, lactate-Ringer's solution was given intravenously with prompt recovery. Advice was given regarding foods liable to cause trouble at this altitude. On January 10, 1943, Feb. 5, Mar. 24, July 9, and August 28, 1943, this boy was seen because of abdominal pain and nausea and severe hives. These attacks always responded to temporary omission of milk, eggs, chocolate and orange juice. These foods were later tolerated, but only in small amounts. He had never had any hives or gastrointestinal disturbances from these foods in New York City. He went to New York and stayed from September to November, 1943, and ate everything without having hives or abdominal pain.

J. D., male, born June 25, 1930. November, 1939. Boy was seen first due to severe hives with secondary infection. By means of carefully followed elimination diet, eggs were found to cause hives on three occasions and were then omitted from the diet. Chocolate was

found to cause abdominal pain, nausea and vomiting, and later hives on various occasions, but was eaten at parties from time to time. On trips to Acapulco (sea level) he ate these foods daily without trouble. In September, 1943, he went to Boston to boarding school, where he was advised to try these foods and note results. He reported in February, 1944, that he can eat everything and has no trouble with hives or digestive difficulties.

T. E., female, born September, 1938. At three months of age, this patient developed a rash and vomiting. She was taken by plane to Brownsville (sea level), a three hours' trip, and recovered immediately on arriving at sea level. She goes to Brownsville for two months every six months and eats all foods, without any hives or gastrointestinal disturbance. Here in Mexico City by keeping a diet record it has been found that orange juice, chocolate, egg, and milk if taken in too large quantity, all cause hives, and the hives subside on omitting these foods. This has been recorded on the written record various times.

D. F., female, 13 years old, was seen a few days after arrival in Mexico City because of infected hives, abdominal pain, and diarrhea. She had never had hives in England where she had lived before coming here. The hives began on arrival and became worse until she omitted orange juice, chocolate, eggs, and milk from her diet, when they cleared up. The exact food involved was not ascertained, as she left shortly after this.

J. G., female, born July 31, 1936, has hives frequently here. These clear up completely in Brownsville and Acapulco. Skin tests in 1939 were positive for oatmeal and rice. In 1943, thirty scratch tests were negative except oatmeal. Rice in spite of being negative has caused a cough on three different occasions. Oranges cause a cough and running nose. These foods were taken without trouble in Brownsville.

S. P., female, born July 17, 1939, was seen July 21, 1941, because of infected hives. She had had hives since 1½ years of age. She was put on an elimination diet, foods being added gradually. On Sept. 18, 1941, skin tests showed egg slightly positive, wheat negative. Nov. 2, 1942. History to date showed that oatmeal caused hives immediately, and that chocolate given for three days caused hives. Eggs caused hives within one day on various occasions. Emotional upsets were observed related to hives, and temper tantrums and hives often occurred together. Feb. 10, 1944. Foods that cause trouble here (eggs, chocolate, peanuts, oatmeal) have been given in Acapulco without any trouble on three different trips. Eggs are given every day in Acapulco with no ill effects. The child breaks out in hives on returning to Mexico City.

C. P., male, born April 21, 1937. This child has had abdominal pain and vomiting related to eggs on four occasions, with prompt subsidence of symptoms on omitting egg from diet. He can eat eggs every day in Acapulco.

T. P., born 1937. This patient was first seen June 17, 1942, at the age of five and a half years with the chief complaint of hay fever especially after his afternoon nap. He has hives here but not in Los Angeles, California. From October, 1941, to May, 1942, he was in Los Angeles and free from hay fever, sneezing, and hives, which are frequent complaints in Mexico City. Intradermal skin tests showed marked reactions to both feathers and house dust, with orris root negative. The mother was advised to cover the pillow with waterproof covering and keep a diary of contacts. On June 24, after covering the pillow, he had improved so that he no longer had sneezing or running nose after his nap, but there still persisted some inflammation of the conjunctivae. Tuberculin test was negative and blood count was as follows: White blood cells 4,750, red blood cells 4,780,000, hemoglobin 78, polys 62, stabs 5, lymphs 20, monos 13. Oct. 19, 1942, his mother reports that he is having hives only every three to four weeks. On Dec. 27, 1942, she reported that oranges were known to cause hives on several occasions. On Oct. 6, 1943, the boy had diarrhea and severe hives, which cleared up with dietary restrictions.

S. P., male, born Mar. 6, 1936, and seen first Sept. 5, 1940, shortly after arrival, because of loose bowel movements, from three to four daily for four days. He recovered promptly on a diet of rice gruel, tea, and gelatin. On Oct. 12, 1940, he had vomiting and abdominal pain preceded by several loose bowel movements and running nose for several days. Pain was intermittent but severe, abdomen showed slight splinting, and rectal examination was painful. At noon the blood count was W. B. C. 6,550, polys 48, stabs 18, lymphs 24, monos 10. At 6 P.M. the blood count was W.B.C. 9,125, polys 32, stabs 16, lymphs 40, monos 12. At 6 P.M. the boy was much better and on Oct. 13, the temperature was normal and he had no

pain. On December 8, 1940, he had vomiting without fever. Since arrival the boy had had frequent abdominal pain occurring about every week, and colds every month lasting from one to two weeks; therefore he had been half sick most of the time. Dec. 11, 1940: Patient vomited five times during the night, and had no fever. Dec. 12, 1940: He was put on a Rowe elimination diet, and foods gradually added. Jan. 2, 1941: Progress was reported as good. Feb. 3, 1941: His mother said that he had had six weeks without vomiting, pain, or running nose. His hemoglobin at this time was 11.5 Gm. June 17, 1941: Summary from diet records showed that he had along very well except when eating chocolate, egg or orange juice. Sept. 8, 1941: The child had vomiting without fever. He had eaten both egg and orange juice, fed by a new servant. Nov. 17, 1941: Patient had asthma for the first time. Nov. 25, 1941: Patient was tested intradermally with eight inhalants and found to have a marked reaction to cats, and a moderate one to kapok and feathers. Dec. 1, 1941: Patient developed mumps, and recovered normally. Mar. 1, 1942: Patient had a severe spell of vomiting without fever, acetone in urine ++, considered due to eating chocolate. Apr. 21, 1942: First dose, 1 c.c., of tetanus toxoid was given. Apr. 22, 1942: The boy developed severe asthma and was given adrenalin. May 7, 1942: Asthma recurred, but was less severe. Intradermal tests for feathers and house dust were repeated, and the reactions were considered to be sufficiently increased to warrant desensitization which was begun and carried out until he left in July, 1943. May 27, 1942: The boy was beginning to gain weight and his nose had stopped running. June 9, 1942: He began to lose weight again. July 10, 1942: Patient had vomiting, headache, sneezing, and nose runing copiously. On July 14 he was taken off of milk and Aug. 7 had gained some weight again. August 17 he had a mild attack of asthma. Sept. 10, 1942: The boy had asthma developing after a bad cold and cough of five days' duration. He had a marked reaction to ragweed. On Oct. 19, 1942, he had sneezing and coughing, and no more trouble until Dec. 2, when he had mild asthma and running nose, with slight hyperemia of the right tympanic membrane, and 101.7° F. Recovery was uneventful.

Until April 15, 1943, he had a period of improved health, with, however, no gain since September. At this time he drank chocolate and on April 16 he had severe headache, nausea and running nose. Enema and milk of magnesia were given and on April 17 he was better. Throughout the history there has been considerable difficulty getting the servants to observe the diet restrictions. On April 22, 1943 he had asthma and running nose, and on May 10 running nose and sneezing. It has been noted that after two or three parties where sandwiches and cake are served, i.e., large amounts of wheat, that he invariably gets a headache. On June 13, 1943 he went to a picnic, ate much bread, and on June 14 had headache, stomach-ache, and temperature 102° F. An enema was given and on June 15 his temperature was normal and he was feeling fine. July 10, 1943: Family left for Quantico, Va. On Dec., 1943, his mother reports that the boy can eat all foods including chocolate without discomfort of any kind, and that he has been in good health with no recurrence of the asthma or headache and vomiting. In other words, he had not had these symptoms before coming to Mexico City, they were marked during his stay here of nearly three years, and he did not have them after returning to sea level.

M. P., male, born June 9, 1939, younger brother of preceding case, was seen first Feb. 3, 1941. On Feb. 27, 1941, he had roseola infantum and on June 17, 1941, a scarlatiniform rash on body, slight amount on face and none on the extremities, without fever. On Sept. 4, 1941, he had hives. On April 1, 8, and 15, 1942, he was given typhoid vaccine. April 27, 1942, he developed cough and running nose, at which time intradermal skin tests were done with a marked reaction to orris root, a mild reaction to feathers and a negative reaction to house dust.

On May 8, 1942, he had severe hives and was placed on an elimination diet, which however was not carefully followed. On May 15 he had more hives and on May 19 the hives continued and he had a running nose. On June 9 he had a mild attack of asthma. Twenty scratch tests were done, including both foods and inhalants. He had positive reactions to milk, rye, peas, horse and goat dander, orris root, kapok and feathers. By June 16 he was better, and he was given the second tetanus toxoid injection on June 23 with no ill effects. He continued to improve and by July 12 evidence indicated that bread caused the hives. The rest of his history is uneventful except for an upper respiratory infection in October with follicular

tonsillitis from which he recovered normally, frequent complaints of stomach-ache in January, 1943, and a cough and running nose in April. This boy got along better than his older brother, S. P. He was considered to be potentially allergic and measures were instituted to protect him, from the beginning, by means of a dust free room, avoidance of foods commonly causing trouble, and varying the diet as much as possible. However his condition was not ideal, and he had allergic reactions from time to time until going to Quantico, Va., where he had none in spite of eating everything, and taking no particular precautions. Like his brother, he had been well until coming to Mexico City, and was well again after going to sea level.

P. C. S., female, born July 31, 1936, and seen shortly after arrival from sta. Engracia (altitude 800 feet) on Aug. 11, 1942. This child had never had hives before coming to Mexico City; they began about two weeks after arrival, and were accompanied by anorexia. On examination a running nose, inflamed conjunctivae and severe hives were found. She was put on an elimination diet. By Aug. 18 the skin was clearing, but the conjunctivae were still inflamed. By Sept. 11 enough evidence had been collected to indicate that tomatoes caused the hives and this was later corroborated by a report on Oct. 20, at which time the skin was clear except for a small patch of eczema on the left cheek and eyelid. She had a positive reaction to feathers and was advised to have her pillow covered with waterproof material. She was seen again Jan. 22, 1943, at which time the conjunctivae were better and it was reported that she had had no trouble in the interval.

J. S., female, born Sept. 2, 1941, was 9 months old when first seen, with a history of never having been ill. She arrived in Mexico on June 2, 1942, and became ill June 6 with fever and ten bowel movements in twenty-four hours. The blood count was 9,600 W. B. C., 28 polys, 17 stabs, and 55 lymphocytes, and the stool culture was negative. On June 8 the count was W. B. C. 6,800, polys 28, lymphs 49, monos 6, and stabs 17. Sulfaguanidine was given, to which the response if any was slow. She was given powdered lactic acid milk, gradually increasing from a weak formula. On July 10, 1942, she had four loose bowel movements which were considered due to liver soup.

On August 1 she again had diarrhea, eight bowel movements in twenty-four hours. No treatment was given except to take her off milk and in 48 hours her bowel movements were two per twenty-four hours. She was then given Sobee which agreed with her for about two weeks. On Aug. 16 she lost her appetite, and had a running nose and cough. Otitis media developed on Aug. 20 and she had fever and a slight rash. She was started on sulfadiazine and nose drops. There was no improvement, the fever and rash continuing, to which was added marked flatulence. On Aug. 24 Sobee was discontinued, and on Aug. 26 the baby had normal temperature and the rash had disappeared. Urine was negative, and W. B. C. 10,750, polys 13, lymphs 87. By Aug. 27 she had a good appetite and her cold had entirely cleared up, W. B. C. 13,050, polys 13, lymphs 86, bas. 1. In September she had fever and a running nose for two days. Urine was negative. In October she had diarrhea again with fever of 102.5° and vomiting. There were 9 bowel movements in the first twenty-four hours, on Oct. 11. This cleared up on a restricted diet. On Oct. 14 in spite of a normal temperature she had nausea and vomiting for one day. The urine was negative and the blood count was as follows: W. B. C. 10,300, polys 21, lymphs 77, monos 2, hemoglobin 14.5 Gm. Her health was good until Feb. 6, 1943, when she had a severe but short illness, with fever ranging between 104° and 105° F. for two days. The physical examination was negative except for eczema in the popliteal spaces. Urine was negative, chest x-ray was negative and there was no blood or pus in the bowel movement. The blood counts are given below:

Feb.	6	W. B. C. 27,750	polys 46	stabs 22	lymphs 29	monos 3		
	8	W. B. C. 32,750	polys 34	stabs 14	lymphs 49	monos 3		
	9	W. B. C. 11,800	polys 17	stabs 11	lymphs 70		Eo 2	
	10	W. B. C. 7,350	polys 16	stabs 1	lymphs 81	monos 1	Eo 1	

In view of her history, after finding no evidence of infection, every effort was made to clear out her gastrointestinal tract by means of frequent enemas and milk of magnesia. Aspirin did not bring down the fever during the first day and she had one convulsion. Sulfadiazine was given, 1 grain per pound per day, and a blood culture was considered but was not done when the temperature began to drop quickly and stay down. On Feb. 9 the child had a normal temperature and was feeling well. On February 5 the child had had corn starch

pudding made with milk, two large helpings. This was the first milk she had had since August, 1942. No explanation was found which could adequately explain this illness.

Shortly after this she was taken to Los Angeles, Calif., where she took all foods including milk and had no trouble or illness. She was advised to do this, but five days before return to Mexico City she was to go on a diet omitting the foods known to cause trouble here, i.e., lamb, liver, eggs, and milk. On May 4, 1943, she returned from California. She had no trouble until May 22, when she had a temperature of 104.5° F., and much fetid gas. On May 21 she had eaten cakes made with milk and egg, the first time she had broken the diet since her return. The blood count was: W. B. C. 15,850, polys 35, stabs 3, lymphs 58, monos 4, Treatment was milk of magnesia and enema and on May 23 she was well. Desensitization to milk was begun very gradually in June, 1943. Wheat was still omitted because of activating the eczema. In December, 1943, the child could take milk, but not wheat. Her condition at this time was good.

M. S., female, born February, 1932, and seen first Feb. 24, 1941. There was a history of colds about every month, recurrent otitis media about fifteen times with mastoid involvement twice. Teeth were carious. Tuberculin test was positive, and chest x-ray negative. Tonsillectomy was done at 18 months of age. The only history of allergy was occasional hives resulting from "cold" injections. Physical examination showed a pale, undernourished nine-year-old girl. On March 10 hemoglobin was 14 Gm., W. B. C. 11,100, R. B. C. 4,930,000, polys 40, Eo 8, lymph 50, monos 2.

April 24, 1941. Stool examination showed cysts of *Endomeba histolytica*. Treatment was with vioform and carborsone, after which stools were negative. July 16, 1941. Patient was seen because of vomiting and hives, no fever. This subsided on the usual treatment of enema, milk of magnesia, and temporarily restricted diet. On Sept. 30, 1941, sedimentation rate was normal and hemoglobin was 15.5 Gm. On Oct. 14, 1941, she had asthma for the first time. Skin scratch tests of 17 inhalants showed 23 foods negative, and cat hair, feathers and house dust markedly positive. Treatment was by means of elimination diet and dust-free room, with plans to desensitize her to feathers and house dust on her return from Acapulco. She improved on this regime. From Nov. 25 to Jan. 28, 1942, she was in Acapulco, sea level, where she ate everything, wheat, eggs, etc. Asthma developed on the trip up to Mexico City. On Feb. 10 she again had asthma, which was thought related to exposure to feathers, and she was advised to have the pillows covered. This was done. The asthma recurred Feb. 21, 23, and 28, but was milder. On Mar. 12 she had asthma and a cough as well, so on March 16, 1942, desensitization to feathers and house dust was begun and continued to date because of the fact that much of the furniture in the house is upholstered with feathers. On Apr. 11 she had a pain in her hip, back and shoulder without fever or swelling. April 16. Patient began eggs. On Apr. 23 she began to vomit, and had no fever. She was given an enema and milk of magnesia and told to omit eggs. The next day she was slightly better although feeling very weak, and on Apr. 25 she was well.

May 5, 1942. Typhoid injection caused temperature of 100.4° F. and malaise for three days. Other doses were given in divided amounts. The diet record showed that grapes had caused hives on several occasions. Her general condition was improving. On June 25, she had vomiting again without fever. After an enema, milk of magnesia, and omission of the latest addition, lamb, to the diet, she recovered and on June 26 was well. On July 4, 1942, she broke the diet and had hives the next day, and asthma on July 6. Raisins and grapes were known at this point to cause anorexia and hives. On October 25, 1942 she had sneezing and mild asthma which cleared on omitting milk.

From Dec. 5, 1942, until Feb. 10, 1943, she was in Acapulco for school vacations. She had no trouble going down but on coming up her nose began to run, with severe sneezing, which developed into asthma on arrival in Mexico City. She was advised to take an enema, milk of magnesia, and go back to the original diet for a few days. On Feb. 11 she was well and went to school. From Feb. 11, 1943, she has had regular desensitizations and diet restrictions except when in Acapulco, and has had no illness except influenza Nov. 18, 1943. From Dec. 3, 1943, until February, 1944, she was in Acapulco and ate all foods without restrictions or ill effects. She had no trouble going down but on coming up she had a running nose and sneezing.

E. S., female, born Mar. 29, 1941, began to refuse her milk Oct. 14, 1941. In November she was still taking less milk and losing weight. Sobee was tried but she would not take it. In January, 1942, she developed eczema on her elbow which improved with tar ointment. In April she had a mild diarrhea with green bowel movements, three in twenty-four hours; this cleared up with a temporarily restricted diet. On May 4, 1942, she had a running nose. July 7, 1942. The baby developed a patchy rash on the buttocks which was thought to be due to Monilia. The diapers were boiled, and gentian violet was used. This gave no results so modified Whitfield's ointment was tried. Treatment was given over three weeks during which time the condition was getting worse. On Aug. 6 the mother was told to omit milk and wheat and two days later the skin was nearly normal, and continued to improve. On Aug. 18 the baby was getting Sobee, but this was discontinued on Aug. 21 and Klim was tried again. On Aug. 26 the baby had a running nose, anorexia, was very fussy and had not gained weight. On Aug. 27 she was better, Klim having been omitted and an enema given, and on Aug. 28 she was much better.

On Oct. 28, 1942 she had vomiting and three bowel movements, and had had no appetite for five days. At this point the baby was taken to Brownsville for two months and there had no trouble at all in spite of taking all foods, and drinking milk. She was advised to go on the restricted diet five days before her return; this was done. However, her mother felt that since she could take milk in Brownsville so well she would try a little here. She gave evaporated milk; this produced urticaria. Several days later, Feb. 27 and 28, Klim was given, and she developed vomiting, acidosis, and temperature of 101°. She was still vomiting Mar. 1, and had a generalized urticaria involving face, body, and extremities, which was severe. Her temperature went to 102.2° on Mar. 2 and became normal on Mar. 3. Treatment was the usual one of clearing out the gastrointestinal tract, and intravenous lactate-Ringer's solution. Blood counts were as follows:

Mar. 3 W. B. C. 15,400, R. B. C. 4,000,000, Hb 11 Gm., polys 43, stabs 4, lymphs 40, monos 9, Eo 3, B 1

9 W. B. C. 10,600, Hb. 12 Gm., polys 30, lymphs 65, monos 3, Eo 2

No milk, eggs, orange juice or chocolate were given from then on, and vitamin C and calcium were added. The mother was very careful to vary the diet, alternating foods so that no one food was given more than once or twice a week, and no food was taken in any great quantity. On this regime the baby improved steadily and gained weight.

On Nov. 11, 1943, she went to Brownsville and her mother was instructed to give foods omitted here, but to begin the Mexico City diet five days before return. On Feb. 18, 1944, her mother reports that in Brownsville she is taking pasteurized cow's milk, egg, bread, orange juice, in fact a diet without any restrictions, that she has been in excellent health and has gained seven pounds.

B. V., male, born Mar. 4, 1942, had had no illness before coming to Mexico. He was seen shortly after arrival Oct. 1, 1942, because of a running nose. On Oct. 21 he began to vomit, and his mother was advised to omit egg and orange juice from the diet, and to continue with evaporated milk. As he vomited frequently on Oct. 28, he was changed to Klim. Nov. 2 he had a running nose again and Nov. 23 anorexia and loose bowel movements, two a day. On Dec. 3 this developed into diarrhea with six bowel movements a day, which however were not liquid. On omitting all milk, the diarrhea cleared up. Dec. 23 he had another spell of running nose, and the same on Jan. 15, 1943. On Jan. 21 he took milk half strength for the first time since December and cried from 1 to 7 A.M. with colic and fever. Then he began to vomit and became acidotic, urine being positive for acetone and negative otherwise. Intravenous lactate-Ringer's solution was given, and the gastrointestinal tract was cleared out. On Jan. 23 he was well, and was started on a milk substitute of cereal, gelatin and oil, which he took well. On Feb. 10 he had his second combined diphtheria and tetanus toxoid injection with no reaction. On Feb. 18, 19, and once the morning of Feb. 20 he was given milk. He developed fever, vomiting and acidosis, and was treated with milk of magnesia, enemas, and subcutaneous fluids. The urine became free of acetone on Feb. 23. His mother was advised to give no milk of any kind for several months. On Apr. 10 he had fever, which lasted three days. Tuberculin test was negative. On Apr. 13 a Schick

test was done. He got along very well until May 18, when milk was given by mistake. His temperature went to 103° F., the fever lasting one day. Treatment was milk of magnesia and enema. On May 26, desensitization to milk was begun by giving very small amounts and gradually increasing. He was not able to take this, but could take boiled goat's milk on which he gained weight. In June, 1943, he went to Laredo, and his mother was advised to feed him everything while there, but to give the Mexico City diet three days before return. He took orange juice, eggs, evaporated cow's milk while there and was very well during the eighteen days. Shortly after his return in July he had three days of unexplained fever, and from then on was very well except for influenza in December, 1943.

A. W., female, born Sept. 11, 1939, was seen Sept. 30, 1943, four days after arriving in Mexico City from West Virginia. Hives began Sept. 28 and got progressively worse. She was treated with enema, milk of magnesia and omission of milk, eggs, orange juice and chocolate from the diet. On Oct. 1 she was better. On Oct. 7 she took orange juice and did not develop hives. On October 13, she ate eggs and had hives. On Jan. 7, 1944, she was in for a check up, and her mother stated that four times hives have been noted directly related to the ingestion of eggs. This child had never had hives before coming to Mexico.

D. Y., a 15-year-old girl was seen first Feb. 21, 1944, because of severe hives and angioneurotic edema. She had lived in Chicago, came down by train to the border and at Brownsville took the plane to Mexico City. She had never had any hives, hay fever, eczema or other allergic complaint in her life, though her father had eczema and her uncles hay fever. Within half an hour of travelling in the plane she was itching all over and by the time she arrived here, both eyes were swollen shut. Since arrival Dec. 16, 1943, she has been much troubled with hives and swelling, except when she went to Acapulco and got complete relief. On her return to Mexico City the hives and angioneurotic edema recurred. She was put on an elimination diet by another physician, but has not had time to arrive at conclusions as to what is causing the trouble. She is going to Acapulco again today, Feb. 22, 1944.

In addition to these cases, I should like to quote the statement of Dr. W. L. Garnett, U. S. Public Health physician here in Mexico City who says he frequently has adult cases, tourists to Mexico City, who complain of severe hives and who have never had hives before in their lives.

The high incidence, the accumulative period, and the character of the symptoms of these proved allergic manifestations in Mexico City are similar to those of mountain sickness. In that condition the well-known symptoms are nausea, vomiting, depression both mental and physical, and diarrhea. Moreover, experimental exposure to reduced partial pressures of oxygen has shown an accumulative period before symptoms appear.[2] At a four-hour daily exposure to a simulated altitude of 12,000 feet, mild symptoms appeared during the third week. With a daily exposure of seven hours, nausea, loss of appetite, etc., appeared at the end of the first week.

It has occurred to me that the anoxia experienced at an altitude such as that of Mexico City may result in a greater absorption and accumulation of protein products than at low levels. Something of this character may account for the very much higher incidence of allergic manifestations in Mexico City. In mountain sickness itself, similar factors may be possible contributing causes. Low partial pressure is evidently the basic cause of mountain sickness and this definitely modifies various physiological functions.

I have been able to find only one reference in the literature available to me indicating an influence of altitude on allergic manifestations. This is a paper by Kopaczewski and Marczewski[3] on anaphylactic shock, recording the production of convulsions in guinea pigs sensitized to beef serum albumin one month previously. These animals together with normal controls were placed in a

pneumatic caisson at the Bourget airdrome and the atmosphere depressed at a rate corresponding to a rise in the air of 1,000 meters per minute until the equivalent of 10,000 was reached. At the latter figure all sensitized animals were in convulsions (with the exception of those which had gone into convulsions earlier). A slow descent was made. At 6,000 meters (in three minutes) all the animals slowly recovered, the only symptom persisting being a lowered temperature. The controls remained normal except for a slight lowering of temperature.

Loss in weight characteristically accompanies the anoxia of high altitudes and the reasons for this have been assumed to be loss of appetite, restlessness, diarrhea, etc.[4] The loss in weight among members of mountain climbing expeditions has always been high. Of particular significance is the report of Hurtado[5] that native Andean children in comparison with those of similar stock at sea level are underweight.

SUMMARY

From the records of a random sample from private practice of 500 individuals in Mexico City, fifteen years of age and under, about 50 per cent were found to be frankly allergic. Of the five hundred, 167 had either severe hives, urticarial rashes or eczema, and one hundred had gastrointestinal symptoms directly related to the ingestion of certain foods. Evidence indicates that these symptoms are either milder or do not occur at all in these same individuals at lower altitudes. In those coming to Mexico City, a period of about three weeks is usually required in the older children for the effect to accumulate and become manifest in symptoms. It is suggested that anoxia occurring at this altitude may result in a greater permeability of the gastrointestinal tract to offending substances. In view of the similarity in many of the cases to conditions found in mountain sickness, the possibility is suggested that similar factors may be contributing causes in such conditions, and may be of interest in aviation medicine.

Recommendations: Children with allergic reactions to foods do well at this altitude if the following precautions are taken: (1) avoiding overloading on any one food, (2) not giving any one food more than once or twice a week, (3) avoiding eggs, chocolate, and too much cow's milk, wheat and orange juice, and (4) supplementing the diet with vitamins A, D, B complex, C and calcium. If certain foods cause serious trouble, they must be temporarily omitted from the diet. Thus it is concluded that aviators who have symptoms similar to those described or others that cannot be explained except on an allergic basis would do well to take these precautions.

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EFFECT OF HEPARIN ON PHAGOCYTOSIS

Observations on P. Lophurae in the Chick

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EXPERIMENTAL observations have shown that heparin does not affect either the phagocytosis of staphylococci by polymorphonuclear leucocytes or the phagocytosis of particles of India ink by the cells of the reticulo-endothelial system.[1, 2] Both of these studies were made upon the rabbit. Von Jansco[3] observed that heparin when given intravenously to mice prevented the phagocytosis of colloidal particles of gold during an interval of ten minutes. Capell[4] has emphasized a fact that is significant in any study of phagocytosis. That is "the reticulo-endothelial cells of the venous sinusoids are even more indifferent to particulate matter than to soluble dye." There may be a similar selectivity in the phagocytosis of various types of substances by cells of the different organs.

The mechanism of phagocytosis is not fully understood at the present time. Both normal and immune sera aid the process of phagocytosis of bacteria by leucocytes. Wright and Douglas[5] gave the name "opsonins" to the active substance which in their opinion was present in serum and aided phagocytosis. It appears from previous experimental studies that heparin does not affect the relationship of serum to the process of phagocytosis of staphylococci by leucocytes.[1] Few observations have been made upon the effect of heparin on bacteriologic and immunologic processes.

Phagocytosis apparently is a significant process in the defense of the host against plasmodia. This process of phagocytosis occurs in all known species infected with malaria. Kinsely and his associates[6] recently advanced the theory that malarial parasitized erythrocytes are coated with either fibrin or a fibrin-like substance and only these coated erythrocytes are phagocytosed. Furthermore, this fibrin or fibrinlike substance produces a clumping of red blood cells. These masses are phagocytized more readily than individual cells. Kite and Wherry[7] in 1915 suggested "that foreign particles as carbon, carmin granules, etc., are "taken up" by leucocytes because the latter . . . have sticky surfaces."

This paper is a report of our observations upon the effect of heparin on the parasitemia produced by *P. lophurae* in chicks. In this study we concur in the opinion that malarial parasites are removed by a process of phagocytosis. This does not imply, however, that phagocytosis is the only defense mechanism that the body has against plasmodia. Observations are also included in this paper on the effect of heparin upon agglutination of both red blood cells and bacteria.

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The data for this paper were obtained during the time I was a member of the Department of Pathology at the University of Tennessee, Memphis, Tennessee.

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METHODS AND MATERIALS

P. lophurae was the strain of malarial parasite used in these experiments. It had been transmitted through young white Pekin ducks at frequent intervals for approximately two years. Six months preceding the time that this experiment was begun, these parasites were transferred to young chicks and thereafter carried in young chicks.

The chicks with malaria were killed on the third or the fourth day of the disease. The blood was obtained immediately from the heart, mixed with an equal volume of a 2 per cent solution of sodium citrate in normal saline. One half cubic centimeter of this inoculum was injected intravenously into the veins about the ankle. The chicks varied in age from three to ten days. In each experiment, however, an identical group of birds was used for both the experimental and the control observation. A majority of the chicks were white giants. White leghorns and white rocks were also used.

The anticoagulant used in these experiments and referred to as heparin was "Liquaemin." It was supplied by Roche-Organon, Inc., through the courtesy of Dr. R. J. Floody. The following procedure was used: 0.1 c.c. of heparin was injected intramuscularly every four hours. A total of six injections was given. Heparin was injected into the chicks with malaria at different intervals as given in the individual experiments.

One cubic centimeter of blood was removed from the heart of the chicks immediately after they were sacrificed. This blood was placed in a test tube (7.5 × 1.0 cm.). It was observed to determine the time in which clotting occurred. The tubes were kept at room temperature for twenty-four hours. They were carefully inverted at intervals to determine the presence or the absence of clots. The blood from normal chicks clots within an interval of five minutes.

The degree of parasitemia was determined by counting the number of parasites in a given number of red blood cells. Blood for these smears was obtained from the toes. They were stained with a combination of Wright's and Giemsa's stains. The number of parasites in 100 cells was counted. These data were used for the statistical analysis. The number of parasites in 500 red blood cells was used for the graphs. This represents a multiple of five for the actual number of red cells counted. The points plotted upon the graphs represent mean values of the counts.

In the experiments where the effect of heparin on clotted blood was studied 1.0 cubic centimeter of blood was obtained from the chick's heart and placed in a test tube (7.5 × 1.0 cm.). After clotting, 1.0 cubic centimeter of heparin was added and the clot was separated from the wall of the tube by carefully rimming it with a small wooden stick. Tubes were kept both at room temperature and in an incubator at 37.5° C. for 24 hours. The clots were then examined microscopically.

In studying the effect of heparin on the agglutination of red blood cells, types two and three sera were used. A suspension of red cells was obtained from the same blood types. A series of titrations were prepared in which heparin plus an equal volume of saline, was used as a substitute for saline in the control. In a second group of observations, serum was placed on a

glass slide with a suspension of red blood cells of opposite type. After agglutination one to two drops of heparin were added. The heparin was added to the serum in some of these latter observations preceding the addition of the red blood cells.

In observing the effect of heparin on the agglutination of bacteria, an (H) typhoid antigen and immune serum were used. A series of dilutions, 1:10 to 1:10, 640 were prepared. Saline was used as the control diluent and heparin as the diluent in the experimental group. In one series the antigen was suspended in saline, and in a second it was suspended in heparin.

The "t" distribution was used for the testing of significance.*

EFFECT OF HEPARIN ON THE CLOTTING OF BLOOD IN NORMAL CHICKS

A group of eight chicks was given intramuscularly two injections of 0.1 cubic centimeters of heparin at four-hour intervals. Two of these were killed four hours subsequent to the last injection. The blood from each of these failed to clot within 24 hours. Two chicks in this group were killed eight hours following the last injection. The blood from these clotted within a period of 30 minutes. Two chicks were killed 10 hours after the last injection of heparin. The blood from one of these chicks clotted within an interval of 10 minutes while that from the second chick clotted within a period of 20 minutes. The blood from normal chicks clots within an interval of five minutes.

TABLE I

EFFECT OF HEPARIN ON THE TIME OF CLOTTING OF CHICK'S BLOOD*

NUMBER OF CHICKS KILLED	HOURS FOLLOWING LAST INJECTION OF HEPARIN	REMARKS
2	4	No clot formed within 24 hours
2	8	No clot formed within 24 hours
13	11	Clot formed in 30 minutes in 2 chicks
		Clot formed in 60 minutes in 3 chicks
		Clot formed in 24 hours in 2 chicks
		No clot formed in 24 hours in 6 chicks
2	12	No clot formed within 24 hours
2	20	Clot formed in 2 minutes in 1 chick
		Clot formed in 10 minutes in 1 chick
4	36	Clot formed within 3 minutes

*0.1 c.c. of heparin injected intramuscularly every 4 hours for 6 injections. Chicks killed and 1.0 c.c. of blood withdrawn from heart.

A group of 25 chicks was injected intramuscularly with 0.1 c.c. of heparin every four hours for a total of six injections. These chicks were killed at subsequent intervals and the blood was observed to determine the time of clotting. The results of these observations are given in Table I. From these data it is evident that the time in which clotting occurs is delayed during a period of 12 hours following the last injection of heparin. In these chicks there is little if any variation from the normal in the time of clotting of the blood removed 20 hours following the last injection of heparin. Blood removed 36 hours subsequent to the last injection of heparin clots within the same interval as that from the controls.

*I am greatly indebted to Dr. F. L. Roberts, Professor of Preventive Medicine, University of Tennessee, for his assistance in the statistical problems concerned in this study.

A retraction of the clot was present, after 24 hours, in the blood of three of the four chicks killed 36 hours after the last injection of heparin. Retraction of the clot did not occur in any of the blood removed from the chicks given the heparin and killed at shorter intervals than 36 hours. In some of these experiments it was observed that the serum overlying the cells was coagulated and that the underlying cells were not clotted. Infrequently, the red blood cells and the serum formed a homogenous clot without ever showing any settling of the red blood cells.

EFFECT OF HEPARIN ON THE PARASITEMIA WHEN GIVEN IMMEDIATELY AFTER THE INJECTION OF MALARIAL PARASITES

Two groups of chicks were used. The injections of heparin were begun thirty to sixty minutes following the inoculation of the malarial parasites. They were discontinued after a period of 24 hours. The degree of parasitemia is given in Fig. 1. From these data it is evident that the number of parasites in the chicks given heparin is always less than that in the control. These data when analyzed statistically show a significant variation from the normal in the heparinized groups.

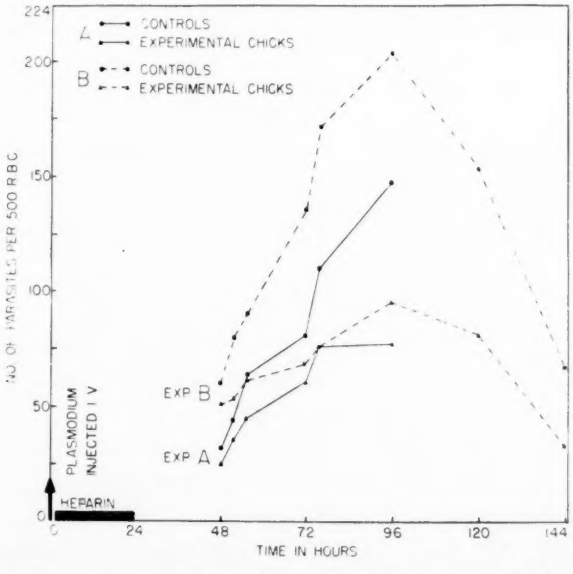

Fig. 1.—Effect of heparin on the parasitemia when given immediately after the injection of malarial parasites. In experiment A seven chicks were used in the experimental group and eight in the control. In experiment B eight chicks were used in each group. The heparin was given over a period of 20 hours in experiment B.

EFFECT OF HEPARIN ON THE PARASITEMIA WHEN GIVEN 24 HOURS FOLLOWING THE INJECTION OF THE MALARIAL PARASITES

In this experiment the parasites were given, and 24 hours later the injections of heparin were begun. They were continued for 20 hours. The degree of parasitemia is shown in Fig. 2. The number of parasites are less in the blood of the chicks given heparin than they are in the controls.

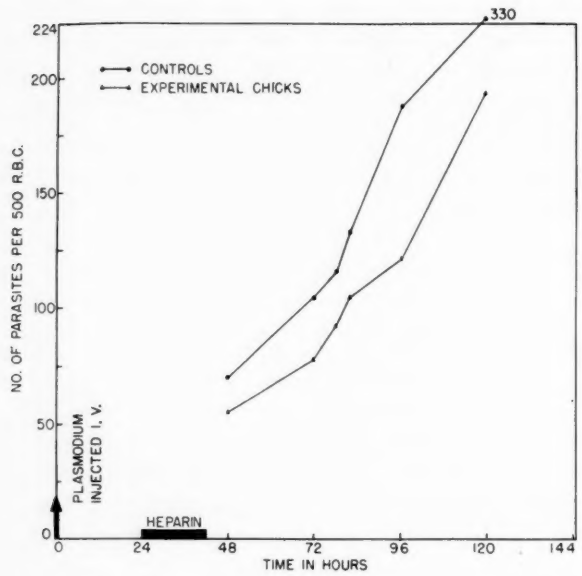

Fig. 2.—Effect of heparin on the parasitemia when given 24 hours following the injection of the malarial parasites. Ten chicks were used in the experimental group and seven in the control.

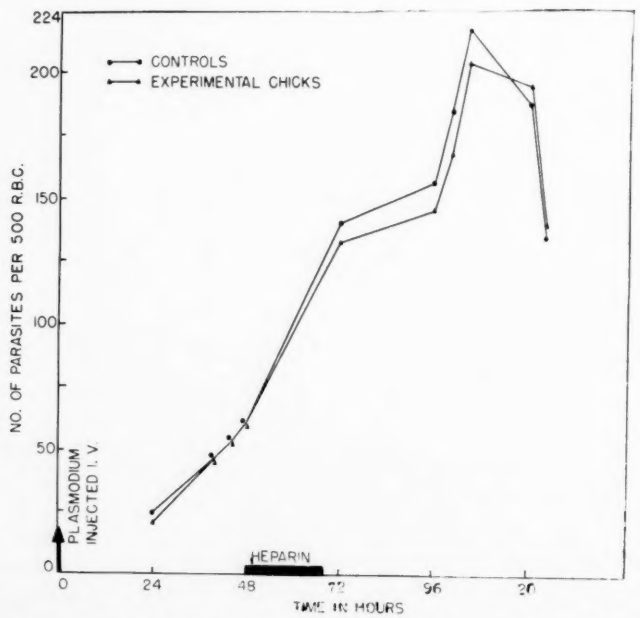

Fig. 3.—Effect of heparin on the parasitemia when given 48 hours following injection of malarial parasites. Fifteen chicks were used in the experimental group and ten in the control.

EFFECT OF HEPARIN ON THE PARASITEMIA WHEN GIVEN 48 HOURS FOLLOWING THE INJECTION OF THE MALARIAL PARASITES

In this experiment the parasites were given intravenously, and 48 hours later the injections of heparin were begun. They were continued for 20 hours. The parasite count in these chicks is shown in Fig. 3. From these data there does not appear to be any variation in the parasitemia in the normal and heparinized chicks. These data when analyzed statistically do not show any significant variation in the heparinized group.

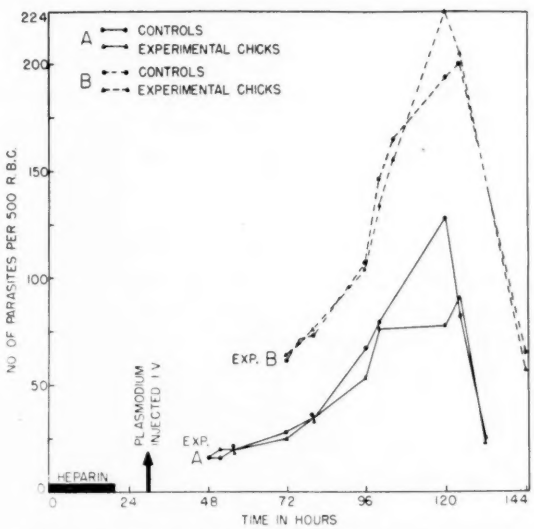

Fig. 4.—Effect of heparin on the parasitemia when given for 20 hours preceding the injection of malarial parasites. In experiment A six chicks were used in each group. In experiment B seven chicks were used in the control group and six in the experimental.

EFFECT OF HEPARIN ON THE PARASITEMIA WHEN GIVEN FOR 20 HOURS PRECEDING THE INJECTION OF THE MALARIAL PARASITES

In the preceding experiment where heparin was given immediately following the injection of the malarial parasites the parasitemia was less than that in the control (Fig. 1). The results of that experiment suggest that the variation could be due to a direct effect of the heparin upon the chick. To study the effect of heparin on the parasitemia further, two groups of chicks were given heparin over a period of 20 hours. The parasites were inoculated 10 hours after the last injection of the heparin. The degree of parasitemia in these chicks is shown in Fig. 4. From these data there is no variation in the degree of parasitemia in the two heparinized groups.

EFFECT OF HEPARIN ON THE AGGLUTINATION OF HUMAN RED BLOOD CELLS BY HETEROLOGOUS SERA

The exact mechanism of the agglutination of human red blood cells by heterologous sera is not fully understood. And, too, the mechanism of the effect of heparin on the clotting of blood apparently is not fully known. Since both of these phenomena may occur in vivo and also be produced in vitro the

effect of heparin on the former is included in our attempt to learn something of the effect of heparin on phagocytosis.

Serum, type 2 and 3, was titrated with heterologous red blood cells. The dilutions ranged from 1:10 to 1:640. There was no variation in either the degree or the extent of agglutination in the group of titrations containing both the heparin and the saline. Observations were also made in which the serum and the cells were placed upon a slide in the manner of the routine typing of blood. Heparin was added to the serum both before and after the addition of red blood cells. In no instance was the agglutination affected. Heparin was added to the agglutinated masses of red cells without producing any macroscopic change.

One may conclude from the present concept of the effect of heparin on the clotting of blood that this anticoagulant would not be likely to affect the agglutination of red blood cells. In the above experimental observation it is shown that heparin, in vitro, does not inhibit the agglutination of human red blood cells by heterologous sera.

EFFECT OF HEPARIN ON THE AGGLUTINATION OF TYPHOID BACILLI

In the preceding experiment it was shown that heparin had no effect upon the agglutination of red blood cells. In this experiment the effect of heparin on the agglutination of the (H) antigen of typhoid was studied. The dilutions of the titrations varied between 1:10 and 1:10,640. Heparin was substituted for saline in one series of these titrations. In a second, a dilution of heparin and saline was used. Saline was used for the control. There was no variation in either the degree or the extent of the agglutination in these three sets of titrations.

EFFECT OF HEPARIN ON CLOTS OF CHICKEN BLOOD

Blood was withdrawn from the heart of normal chicks and 1.0 c.c. was placed immediately in test tubes (7.5 × 1.0 cm.). This blood clotted within an interval of five minutes. One cubic centimeter of heparin was then added to the clotted blood. The clot was separated from the wall of the tube. There was no macroscopic variation after 24 hours in the consistency of the clots when heparin was added or in the controls.

The above procedure was repeated using blood from chicks with a high malarial parasitemia. Heparin, likewise, did not affect these blood clots. It is well known that heparin does not affect a formed thrombus. The present results are consistent, therefore, with similar observations of other investigators. Furthermore, they show that clotted blood, containing malarial parasites, is not affected by heparin.

DISCUSSION

The data in these experiments show that the parasite count is less in chicks given heparin than it is in the controls when the heparin is given within a period of 48 hours following the injection of plasmodia. Furthermore, the data suggest that heparin does not affect the parasite count when it is given in a similar quantity either 24 hours preceding or 48 hours subsequent to the injection of the parasites.

The probable error in this method of computing parasite density is very high. The time at which the counts were made vary in the different groups. Because of these facts the significance of a statistical analysis is limited. It is interesting to note in these data that the number of malarial parasites in chicks given heparin is always less than it is in the controls. There is insufficient evidence, however, at the present time, to determine, first, whether heparin stimulates phagocytosis; second, whether it has an injurious effect either directly upon the parasites, or in some other manner affects the body's defense mechanism against the plasmodium. It is suggested that heparin may affect directly *P. lophurae* since the greatest diminution in the parasite count occurs during the early phase of the infection. When the disease is present for 48 hours and heparin is given there is not a demonstrable variation in the parasite count.

Apparently there is nothing in the present study to indicate that the heparin inhibits the phagocytosis of malarial parasites. This observation may be significant in view of the conclusion made by Kinsely and his associates[6] from their study of *P. knowlesi* infection in monkeys. They state that "a layer of fibrin or a fibrinlike substance is precipitated on knowlesi parasitized erythrocytes. Coated erythrocytes do not stick to ordinary endothelium but do stick to one another, forming "clumps" which stick to and are instantly engulfed by hepatic phagocytes. . . . Ordinary endothelium becomes sticky, too, and solidly coated with leucocytes. Hence leucopenia in drawn malarial blood." Rabinovitch[8] has shown that heparin will prevent the formation of fibrinous thrombi in rabbits given dogs' serum while this anticoagulant does not affect the formation of agglutinated red cells which follow the injection of ox serum.

In the pathological study of the tissue from the same monkeys used by Kinsely and his associates[6] Rigdon and Stratman-Thomas[9] were unable to demonstrate "clumps" in the circulatory system, and likewise, they did not observe that the vascular endothelium was "solidly coated with leucocytes." If these "clumps," as observed by Kinsely, develop as a result of the parasitized erythrocytes being coated with either fibrin or a fibrinlike substance, it is obvious that something has occurred to this hypothecial substance that coats these erythrocytes because there were no clumps demonstrated in the sections. Quick[10] has said "the theory that agglutination and phagocytosis depends on a fibrin coating about the affected particles or cells must include a mechanism whereby this fibrin is removed."

Thrombi and embolic masses from intravascular thrombi are readily demonstrable in paraffin prepared histological sections of tissue. By exerting gentle pressure on the lungs of rabbits that died following the injection of ox serum, Rabinovitch[8] squeezed from the vessels numerous thrombi consisting of agglutinated red cells which were readily seen under the microscope. Only infrequently have masses been observed in blood smears from both man and monkey infected with malaria.[11, 12] Best, Cowan, and McLean[13] have discussed the mechanism of the formation of thrombi and they have shown that heparin inhibits their development. This anticoagulant has no effect upon a previously formed thrombus. Kinsely says that only parasitized erythrocytes coated with fibrin or a fibrinlike substance are phagocytosed. If so, it would appear likely that heparin may prevent the coating of these erythrocytes by fibrin and thus

prevent them from being phagocytosed. The parasite count in chicks given heparin would be increased, therefore, over that of the controls. The data in these experiments do not indicate any inhibition in the degree of phagocytosis in the heparinized chicks.

It has been shown in previous experiments that heparin, when given intravenously to rabbits has no effect on the localization of either polymorphonuclear leucocytes or trypan blue in areas of injury.[1] Furthermore, this anticoagulant does not affect the development of an acute inflammatory reaction in the skin of the rabbit. Heparin, likewise, does not inhibit the phagocytosis of particles of India ink by the cells of the reticulo-endothelial system.[2] To these observations now may be added the observation that heparin has no effect on either the agglutination of human red blood cells by heterologous serum or the agglutination of typhoid bacilli by immune sera. Heparin also has no fibrinoloytic effect on clots of normal and parasitized chicken blood.

There apparently is no evidence from the study of the pathologic lesions occurring in the monkeys infected with *P. knowlesi*, and too, from the study of the effects of heparin on the parasitemia in chicks to support the opinion that fibrin or a fibrinlike substance coats parasitized erythrocytes to produce clumping and to inhibit phagocytosis. Immunologic reactions characterized by agglutination are well known in which fibrin does not occur. Holmes[14] has recently reviewed this problem and discussed the phenomena of agglutination and precipitation in relation to surface tension.

Robert Fahraeus[15] in 1929 reported in man the presence of intravascular aggregation of erythrocytes identical to those described by Kinsely and associates.[6] In this paper Fahraeus reviewed the phenomena of rouleau formation, intravascular aggregation of erythrocytes as observed in disease and the mechanism of the cellular changes as observed in both the normal and in certain pathological conditions.

Many of the normal chicks died during the period in which heparin was injected. Hemorrhages were present in the tissues inoculated and also in the serous cavities. Chicks infected with malaria and given heparin also died during the time of the experiment. They showed hemorrhages similar to those in the control group. This bleeding into the serous cavities of the chick is interesting in view of previous observations upon capillary permeability in rabbits given heparin.[1] In studies in the rabbit it was believed that "heparin has no effect on capillary permeability." It is now obvious from our present observations in the chick that both the type of animal and the quantity of heparin must be considered in studying the effect of heparin on capillary permeability. It would appear from the results of many experimental observations that capillary permeability may be affected by heparin. The use of heparin in clinical cases of acute malaria is contraindicated since hemorrhages do occur in the viscera in this disease.[16] The presence of this anticoagulant may facilitate the escape of red blood cells through the capillary walls and thereby produce larger hemorrhages in the parenchymatous tissues.

CONCLUSION

There is no evidence from this study to support the opinion that heparin inhibits the process of phagocytosis of malarial parasites. The parasitemia is

less in chicks given heparin immediately following the injection of the parasites than it is in the controls.

Heparin apparently has no effect upon either the agglutination of human red blood cells by heterologous serum or the agglutination of typhoid bacilli by immune sera.

Heparin has no effect apparently, on previously formed clots of normal and parasitized chicken blood.

The role of heparin in other of the fundamental biological processes is briefly referred to.

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EFFECT OF GONADS AND ADRENALS ON THE ABSORPTION OF SUBCUTANEOUS SESAME OIL

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MANY plant oils, used as solvents for hormones, are considered to be without effect on the injected subject. Evidence is being accumulated, however, to show that such oils are not bland when injected into certain animals. The oil used may either have various effects on the animal, or hormones may influence absorption of the oil.[1-13]

METHODS

To test the effect of hormones from the animal's own gonads and adrenals on the rate of absorption of subcutaneous sesame oil, the following experiments were performed on adult albino rats in groups of ten animals each. The rats were kept in a thermostatically controlled room and given tap water and a commercial diet (Wayne Dog Blox) ad libitum. One cubic centimeter of sesame oil was injected subcutaneously, as 0.5 c.c. doses, at two sites over the left flank into normal, adrenalectomized, or adrenalectomized-gonadectomized rats (at the time of operation), and gonadectomized animals (2 weeks after operation). All animals were killed fourteen days after injection, except the pregnant ones, which were operated upon and injected on the tenth day of pregnancy, and killed twenty-one days after delivery.

The procedure for recovering the oil was similar to that reported by Turner and Mulliken.[2, 3] The initial weight of 1 c.c. of sesame oil was 915± 4.5 mg.* Bruce and Tobin[4] have reported the composition and other properties of this oil from a similar source. A comparable area of skin and subcutaneous tissue taken from the right flank of each animal was extracted similarly to indicate the amount of ether-soluble matter, other than injected oil, present in the recovered oil. Corrections are made in the data for the weight of this matter.

RESULTS

The amount of oil absorbed by normal animals is proportional to the length of time the oil remained in the animals: a group of normal males killed 30 minutes, and another group killed 24 hours after injection, absorbed 299 ± 58 mg. and 414 ± 99 mg. of oil, respectively. Increasing amounts of oil were absorbed with longer experimental periods: males killed 12 days after injection absorbed 473 ± 187 mg. of oil. Both control and experimental animals were studied, for similar periods, in order to control the time element on the rate of absorption of the subcutaneous sesame oil.

The significance of the differences in the amount of oil absorbed by the various groups was tested by Fisher's "t" method. Only those differences

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*The accuracy of the recovery method employed is indicated by the weight of oil run through the above procedure which varied only by ± 9.2 mg. from the initial weight of the oil.

whose "t" values were 2.10 (P = 0.05) or greater were considered significant and are discussed below. The amount of oil absorbed by the normal and operated animals is shown in Table I.

Effect of Gonads.—Normal females absorbed the greatest quantity of oil, the castrate males and normal males were next in descending order, whereas spayed females absorbed the smallest amount of oil in this normal or gonadectomized group. Significant differences were found in the amounts of oil absorbed by the spayed females compared to normal females ("t" value 2.9),

TABLE I

ABSORPTION OF SESAME OIL FROM THE SUBCUTANEOUS TISSUE OF RATS

Oil absorbed‡	*Normal ♀	*Castrate ♂	*Normal ♂	*Adrenalectomized ♂	*Adrenalectomized ♀	*Spayed ♀	*Adrenalectomized castrate ♂	*Adrenalectomized spayed ♀	†Virgin ♀	†Pregnant adrenalectomized ♀	†Pregnant normal ♀
Per cent	55	52	51	49	42	31	31	25	72	63	51
Avg. mg.	512	479	473	451	386	288	284	233	665	578	467
σ mg.	197	203	187	162	207	143	142	123	124	156	186

*Killed 14 days after injection.
†Killed 31 days after injection.
‡Based on initial weight of 1 c.c. oil, 915 ± 4.5 mg., injected.

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

castrate males (2.4), or normal males (2.5). However, no significant differences were found between the amount absorbed by normal females or by castrate or normal males. There was likewise no significant difference in the oil absorbed by castrate and normal males. The sesame oil injection had no effect on the reproductive physiology of the normal rats, and influenced in no way the atrophy of the accessory reproductive organs resulting from gonadectomy.

Effect of Adrenals.—Although bilateral adrenalectomy appeared to decrease the amount of oil absorbed by rats, this difference was not significant. There was a significant difference only when the amounts absorbed by adrenalectomized males were compared with those absorbed by spayed females (2.4).

Effect of Gonads and Adrenals.—Removal of both the gonads and the adrenals from rats reduced the amount of oil absorbed by such animals. This is shown by the differences in absorption of oil by adrenalectomized-castrate males and adrenalectomized-spayed females, respectively, compared with that of normal females (3.0 and 3.8), castrate males (2.5 and 3.3), normal males (2.6 and 3.4), and adrenalectomized males (2.5 and 3.4). The adrenalectomized-castrate males absorbed 31 per cent while the adrenalectomized-spayed females absorbed 25 per cent, indicating that the ovaries may influence slightly the utilization of sesame oil in adrenalectomized animals. However this latter difference was not significant.

Effect of Normal Pregnancy and Adrenalectomy During Pregnancy.—Since these experiments were of longer duration than the preceding ones (31

days), the amounts of oil absorbed cannot be compared directly with those from the nonpregnant groups. However, as compared with virgin females studied for a similar period, which absorbed 72 per cent of the oil, the adrenalectomized-pregnant females absorbed 63 per cent, and the normal-pregnant females utilized relatively less, 51 per cent. Only 50 per cent of the adrenalectomized-pregnant animals delivered; three of these lactated sufficiently to maintain 63 per cent of their litters until the twenty-first day. A significant difference was found between the amounts of oil absorbed by the virgin females and pregnant normal females (2.8); but no significant difference was found when the data from the pregnant adrenalectomized females were compared with that from the virgin females or normal pregnant rats.

CONCLUSIONS

The rate of absorption of sesame oil from rats is reduced by removal of the ovaries, removal of the adrenals in addition to the gonads, and by pregnancy. The sesame oil was injected subcutaneously in 1 c.c. amounts and recovered later as an ether-soluble extract of the injection sites.

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THE EFFECT OF BILE ACIDS ON THE BILIARY EXCRETION OF NEOARSPHENAMINE AND MAPHARSEN

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IT HAS been reported that the administration of sodium dehydrocholate ameliorates the jaundice and anorexia of arsenical hepatitis even when the arsenical injections are continued.[1] It has also been reported that in rabbits when neoarsphenamine was injected with sodium dehydrocholate, less arsenic was recovered from the liver than when the drug was injected alone.[1, 2] It is well established that arsenic is excreted in the bile following injection of arsphenamines, but the rate of excretion has not been studied in permanent bile-fistula animals.

In view of the extensive use of neoarsphenamine and mapharsen and the occasional occurrence of a clinically evident hepatitis when they are used, it was considered worth while to investigate (a) the rationale of the claim that the administration of dehydrocholic acid has a favorable effect in arsenical therapy and (b) the effect of bile acids on the excretion of arsenic.

Dehydrocholic acid (oxidized cholic acid) may act to prevent the occurrence of an arsenical hepatitis in several ways. (a) The bile acid causes a hydrocholeresis[3] which may augment the excretion of arsenic in the bile and decrease the exposure of the liver to arsenic. (b) Dehydrocholic acid increases the hepatic arterial blood flow[4] which may, through the consequent increased oxygen supply, protect the liver by increasing its metabolism and its ability to detoxify harmful substances. (c) The bile acid may combine with the arsenical in such a way as to render it less hepatotoxic. (d) Since bile salts injected intravenously increase the permeability of the capillaries to certain compounds,[5] the simultaneous injection of dehydrocholic acid and the arsenical may cause more of the latter to pass into the general tissues than otherwise, and thus decrease the exposure of the liver to the arsenical. This would expose other tissues to more of the arsenical, which may or may not be a desirable action depending upon whether the toxic effect on other tissues or the treponemicidal action of the drug was more markedly increased.

This investigation has been confined to the study of the effect of two types of bile acids on the excretion of arsenic in the bile.

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GENERAL METHODS

Two types of bile acids were used. One was conjugated, unoxidized bile acids represented by sodium glycocholate and taurocholate (Bilron, Lilly). The other was an unconjugated, oxidized bile acid represented by sodium dehydrocholate (Decholin, Riedel de Haen, and Ketochol, Searle). The former preparation, which we shall hereafter refer to as "ox-bile" acid, because it was made from cattle bile, causes a moderate choleresis but does not increase the hepatic arterial blood flow. The latter preparation or sodium dehydrocholate causes a hydrocholeresis and increases the hepatic arterial blood flow.

Biliary fistula dogs with a duodenal fistula for the return of bile into the intestine were used[6] for the experiments extending over a week or more. Acute bile fistula dogs under nembutal anesthesia were used for the tests lasting only three or four hours.

The bile was analyzed for arsenic by the method of Cassil and Wichman.[7] This method was reported by them to be accurate for amounts of arsenic varying from 5 to 500 micrograms in the sample analyzed. We obtained the best duplicate checks when the sample contained less than 300 micrograms.

The neoarsphenamine injections consisted of one dose of 300 mg. which contained 60 mg. of arsenic. The mapharsen injections consisted of one dose of 60 mg. which contained approximately 17.4 mg. of arsenic. These doses range from 2 to 4 times the *single* weekly human therapeutic dose on the basis of body weight. However, when mapharsen is used in the intensive treatment of syphilis, from 1.0 to 1.2 Gm. is given in a five-day period.[8, 9] This dose averages 240 mg. daily, and amounts to four times the dose used in this study.

The dogs invariably vomited upon receiving mapharsen. For this reason the drug was always given at least ten hours after a meal. This was done because the amount of cholic acid formed depends on the protein absorbed and metabolized, and in order to maintain the animals under controlled conditions, food intake must be constant.

Arsenical injections were not repeated in the same animal more often than once a week, and the sequence of the tests was rotated so that the animals did not all receive the drugs and the drugs plus bile acids in the same order. The volume output of bile was recorded during the tests, but is not included in the data. The average volume output is not changed significantly by the arsenical injections.[10]

RESULTS

Neoarsphenamine

The rate of disappearance of arsenic from the bile after a single intravenous injection of neoarsphenamine: In this series of tests the animals were fed the standard diet every twelve hours; bile was not returned to the intestine, nor were bile acids given orally. Three hundred milligrams of neoarsphenamine were injected and the twenty-four-hour bile output was analyzed for arsenic.

The results are shown in Table I. Most of the arsenic was excreted during the first twenty-four hours after the injection and relatively small amounts were excreted after seventy-two hours. Traces of arsenic were present in the

TABLE I

SHOWING THE DAILY BILIARY EXCRETION OF ARSENIC FOLLOWING INJECTION OF 300 MG. NEOARSPHENAMINE CONTAINING 60 MG. ARSENIC. NO BILE OR BILE ACIDS WERE GIVEN*

	MG. BILIARY ARSENIC EACH DAY AFTER THE DOSE								
DOG	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS	TOTAL	% OF DOSE RECOVERED IN BILE
C4	18.83	1.88	.82	.72	.53	.58	.75	18.11	31
C4	17.09	15.72	2.81	.66	.62	.46	.29	37.55	63
C7	16.08	4.42	2.10	.49	.93	.63	Trace	24.65	41
D9	19.33	2.22	2.22	.92	Trace	Trace	Trace	24.69	41

*These tests show that most of the arsenic excretion occurs in 72 hours after giving the drug. In subsequent tables arsenic excretion is given for 24- and 72-hour periods following the dose of neoarsphenamine.

bile seven days after the injection. From 27 to 32 per cent of the injected arsenic was recovered in the bile in twenty-four hours, and from 31 to 63 per cent of the injection was excreted in the bile in seven days.

The effect of "ox-bile" acids and dehydrocholic acid on the excretion of arsenic after a single injection of neoarsphenamine: In this series of tests a group of bile fistula dogs were fed the standard diet every twelve hours until the volume and cholic acid output were constant. Three hundred milligrams of neoarsphenamine were injected intravenously. The bile was collected every twenty-four hours for three days and analyzed for arsenic. A seventy-two-hour period was chosen because the bile was relatively free of arsenic after that time.

Twelve dogs (Table II) excreted an average of 24 mg. of arsenic in seventy-two hours, or 40 per cent of the amount injected. This was the control series. When 1.5 Gm. of "ox-bile" acid was fed every twelve hours for three days before and after giving neoarsphenamine, an average of 26.9 mg., or 44 per cent of the arsenic was recovered in the bile. When 1.5 Gm. of dehydrocholic

TABLE II

SHOWING EFFECT OF "OX-BILE ACIDS" AND OF DEHYDROCHOLIC ACID ON EXCRETION OF ARSENIC IN BILE FOR A PERIOD OF 72 HOURS AFTER INTRAVENOUS INJECTION OF 300 MG. NEOARSPHENAMINE CONTAINING 60 MG. ARSENIC

	MG. ARSENIC EXCRETED IN 72 HOURS							
	CONTROL. NO BILE ACID		3 GM. "OX-BILE ACIDS"			3 GM. DEHYDROCHOLIC ACID		
DOG	MG.	% OF DOSE	MG.	% OF DOSE	% CHANGE	MG.	% OF DOSE	% CHANGE
C4	35.5	59.4				20.8	33.6	−42
C7	23.6	39.3				14.8	24.6	−38
D9	23.8	39.6	43.9	73.0	+ 85			
F3	21.2	35.3	22.7	37.8	+ 7	24.1	40.0	+14
F6	18.9	31.4	14.6	24.4	− 23	16.7	27.8	−12
F7	25.0	41.8	26.8	44.6	+ 7			
F8	35.7	59.6	15.6	26.0	− 57	29.4	49.0	−18
F9	17.6	29.4	36.1	60.0	+104			
F10	20.2	33.6	19.7	33.0	− 2	10.4	17.4	−50
G1	23.5	39.2	35.1	58.5	+ 50	28.3	47.2	+21
G5	25.3	42.2	30.1	50.0	+ 19	21.8	36.4	−14
						25.9	43.1	+ 2
G6	17.7	29.6	21.3	36.0	+ 20	14.7	24.4	−17
						19.2	32.0	+ 8
Aver-age	24.	40.1	26.9	44.3	+ 15	20.5	34.1	−16
					+ 12			−15

$$\frac{26.9 - 24.0}{24} = +12\% \qquad \frac{24.0 - 20.5}{24} = -15\%$$

acid was similarly fed, an average of 20.5 mg., or 34 per cent of the dose, was eliminated in the bile in seventy-two hours.

Thus, the administration of "ox-bile" *apparently* increased the excretion of arsenic by an average of 15 per cent as compared with the control series, and the feeding of dehydrocholic acid decreased arsenic excretion by an average of 16 per cent. A statistical analysis of the data, however, revealed that the average increase of 15 per cent and the average decrease of 16 per cent as compared with the control were not significant. Nevertheless, the difference between the arsenic excretion when "ox-bile" was fed and when dehydrocholic acid was fed was sufficiently statistically significant to indicate that it was probably not due to chance variation. This difference suggests only that dehydrocholic acid tends to retard the biliary excretion of arsenic as compared to the excretion when "ox-bile" acids are given.

Comment.—Sodium dehydrocholate and "ox-bile" acids increase biliary volume output on the average by 100 per cent and 35 per cent,[3] respectively. Since, when neoarsphenamine was given, significantly more arsenic was not excreted in the bile when bile acid was given than when none was given, *choleresis, per se, does not augment the biliary excretion of arsenic* administered in the form of neoarsphenamine.

The differences in the excretion of arsenic in the control series and the series given "ox-bile" acids and dehydrocholic acid cannot be attributed to previous arsenic administration. In one-third of the animals the control tests were performed last; in one-third the "ox-bile" acid was given last, and in one-third the dehydrocholic acid was given last. Further, at least one week elapsed between arsenical injections which is sufficient time to clear the bile of measureable amounts of arsenic.

To make certain that the presence of choleresis was not associated with a greater excretion of arsenic during the first 24 hours, the biliary arsenic for the first twenty-four hours of the tests was tabulated. The data shown in Table III show that arsenic excretion in the bile was not increased the first twenty-four hours in the series given either bile acid preparation.

The effect of injecting neoarsphenamine with sodium dehydrocholate on the excretion of arsenic in the bile: Appel and Jankelson[2] found that when a mixture of sodium dehydrocholate and neoarsphenamine was injected into rabbits, less arsenic was found in the liver after two to twenty-four hours than when neoarsphenamine was given alone. This observation was interpreted as indicating that the dehydrocholate caused more arsenic to be excreted in the bile. Our results on the dog do not support such a conclusion. It is possible, however, that different results might be obtained when the bile salt is mixed with the neoarsphenamine in the same syringe rather than giving the dehydrocholate orally. The following experiements were performed to examine this possibility.

Chronic or permanent biliary fistula dogs were so prepared that their collecting tube system contained about 5 c.c. of bile. Such animals cannot be used satisfactorily for tests lasting only an hour or so during which the output of bile may be less than 5 c.c. Therefore, "acute" or temporary bile fistula

TABLE III

SHOWING THE EFFECT OF "OX-BILE ACIDS" AND OF DEHYDROCHOLIC ACID ON EXCRETION OF ARSENIC IN BILE FOR A PERIOD OF 24 HOURS AFTER INTRAVENOUS INJECTION OF 300 MG. NEOARSPHENAMINE CONTAINING 60 MG. ARSENIC

DOG	MG. ARSENIC EXCRETED IN 24 HOURS							
	CONTROL: NO BILE ACID		1.5 GM. "OX-BILE" ACIDS			1.5 GM. DEHYDROCHOLIC ACID		
	MG.	% OF DOSE	MG.	% OF DOSE	% CHANGE	MG.	% OF DOSE	% CHANGE
C4	17.09	28.5				12.39	23.0	−33
C7	16.08	26.8				11.65	19.4	−28
D9	19.33	32.2	29.98	50.0	+ 55			
F3	16.77	28.0	16.45	27.4	− 2	16.50	27.5	− 2
F6	11.37	19.0	9.15	15.3	− 18	10.10	16.8	−11
F7	17.58	29.3	19.65	32.8	+ 12			
F8	25.80	43.0	9.63	16.0	− 65	17.50	29.2	−32
F9	11.80	19.7	26.85	45.0	+128			
F10	14.50	24.2	13.85	23.0	− 3	5.64	9.4	−63
G1	15.95	26.6	25.20	42.0	+ 58	17.90	29.8	+12
G5	17.53	29.3	22.02	36.6	+ 25	16.78	28.0	− 4
						15.78	26.3	−10
G6	11.22	18.7	13.80	23.2	+ 23	12.10	20.2	+ 8
						10.01	16.7	−10
Average	16.25	27.1	18.66	31.1	+ 21	13.30	22.4	−16
					+ 14			−18

animals were prepared under nembutal anesthesia with the cystic duct clamped and a short collecting tube placed in the common bile duct.

The control series of dogs received 300 mg. of neoarsphenamine alone intravenously. A second series were given an injection of mixture of 300 mg. neoarsphenamine and 5 c.c. of 20 per cent sodium dehydrocholate. To this series additional injections of 5 c.c. of sodium dehydrocholate were given hourly. A third series of dogs received a single injection of dehydrocholate to induce a brisk choleresis, and ten minutes later were given 300 mg. of neoarsphenamine.

TABLE IV

SHOWING THE EFFECT OF INTRAVENOUS SODIUM DEHYDROCHOLATE ON THE BILIARY EXCRETION OF ARSENIC DURING THREE HOURS AFTER AN INJECTION OF 300 MG. OF NEOARSPHENAMINE CONTAINING 60 MG. OF ARSENIC*

DOG	WT. KG.	ONE HOUR		TWO HOURS		THREE HOURS		TOTAL			PROCEDURE
		VOL. IN C.C.	MG. AS.	VOL. IN C.C.	MG. AS.	VOL. IN C.C.	MG. AS.	VOL. IN C.C.	MG. AS.	% REC.	
1	12.0	1.4	0.16	5.6	1.75	5.6	1.12	12.6	3.12	5.0	Series I. Controls; no bile acid
3	11.2	6.6	1.57	6.8	1.70	5.8	1.46	19.2	4.74	7.8	
4	9.5	4.8	1.54	5.4	3.20	6.8	3.37	17.1	8.13	13.5	
Ave.		4.3	1.10	5.9	2.20	6.1	2.01	16.3	5.33	8.8	
2	11.0	14.5	Trace	10.6	0.24	19.2	0.36	44.3	0.60	--	Series II. Mixture & 5 c.c. 20% sod. dehydrocholate each hour
5	8.4	15.5	0.23	14.2	0.55	12.5	0.23	32.2	1.02	--	
Ave.		15.0	0.12	12.4	0.40	15.9	0.30	38.3	0.81		
6	9.7	25.5	2.70	22.5	6.09	4.5	0.90	52.5	9.69	16.0	Series III. One injection sod. dehydrocholate 10 minutes before neo. injection
7	8.0	23.5	0.22	11.2	0.94	8.5	0.86	43.2	2.02	3.3	
8	6.6	14.2	0.60	11.0	1.15	8.6	0.83	33.8	2.58	4.3	
Ave.		21.1	1.17	14.9	2.73	7.2	0.86	43.2	4.76	7.9	

*Additional experiments by De Hoog and Gutmann in this laboratory confirm these findings.

The results are shown in Table IV. The choleresis produced by dehydrocholic acid did not increase the excretion of arsenic. This confirms the foregoing results from chronic biliary fistula dogs. The *simultaneous injection of sodium dehydrocholate and neoarsphenamine resulted in a marked decrease in the biliary excretion of arsenic.* These results indicate that the decreased retention of arsenic in the liver of rabbits when dehydrocholic acid was administered with neoarsphenamine observed by Appel and Jankelson was probably not due to increased excretion of arsenic in the bile. More likely, increased amounts of the arsenical passed into other tissues and this decreased the quantity of arsenic entering the liver.

Mapharsen

The effect of dehydrocholic acid on the excretion of arsenic in the bile after an intravenous injection of mapharsen: Table V presents the data from eighteen tests on ten dogs which were performed similarly to the experiments with neoarsphenamine. Sixty milligrams of mapharsen, containing 17.4 mg. of arsenic, were given to one series of dogs without feeding bile acids. To another series, 1.5 Gm. dehydrocholic acid was fed every 12 hours for 3 days before and after injecting the arsenical. Since it was found in confirmation of the work of Eagle and Hogan[19] on rabbits that the excretion of arsenic in the bile after the administration of mapharsen was practically complete in forty-eight hours, Table V shows the biliary arsenic for this two-day period.

TABLE V

SHOWING EXCRETION OF MAPHARSEN IN BILE FOR A PERIOD OF 48 HOURS FOLLOWING INJECTION OF 60 MG. OF THE DRUG CONTAINING 17.4 MG. ARSENIC. EIGHT TESTS SHOW THE RESULTS WHEN NO BILE ACIDS WERE GIVEN. TEN TESTS SHOW EFFECT OF DEHYDROCHOLIC ACID, 1.5 GM. EVERY 12 HOURS ON EXCRETION OF ARSENIC

DOG	NO BILE ACID			DOG	DEHYDROCHOLIC ACID		
	MG. ARSENIC IN 48 HOURS	PER CENT OF DOSE	% CHANGE		MG. ARSENIC IN 48 HOURS	PER CENT OF DOSE	% CHANGE
F3	7.90	45.4		G5	7.06	41	
F8	8.92	51.2		G5	4.92	28	
G1	9.32	53.5		G6	10.40	60	
G1	9.74	56.0		G6	9.16	53	
G2	6.87	39.5		G8	6.21	36	
G3	10.03	57.5		G8	6.01	34	
G5	5.47	31.4		G9	4.94	28	
G6	5.84	33.6		G9	5.72	33	
				G10	5.07	29	
				G10	5.26	30	
Ave.	8.02	46.0	–		6.47	37.2	–19

The results in Table V show that the administration of dehydrocholic acid was not associated with an increase in arsenic excretion, though the bile acid had a marked choleretic action. In fact, the excretion of arsenic was 19 per cent less on the average than that observed in the control series when no dehydrocholic acid was given. Although this difference is not statistically significant, the tendency for dehydrocholic acid to decrease excretion of mapharsen-arsenic is similar to that observed for neoarsphenamine-arsenic.

It is worthy of noting that the percentage recovery of mapharsen-arsenic at forty-eight hours (Table V) is approximately the same as that of neo-

arsphenamine-arsenic at seventy-two hours (Table II). This shows that mapharsen-arsenic is more rapidly excreted in the bile than neoarsphenamine-arsenic.

The total excretion of neoarsphenamine arsenic: Several reports indicate that arsenic is excreted in the feces and to a lesser extent in the urine.[11-13]

In one biliary fistula dog the amount of arsenic excreted in the bile and in the urine was determined after injecting 300 mg. of neoarsphenamine. In this experiment 65 per cent of the arsenic was recovered in the bile and 30 per cent in the urine after seventy-two hours.

Is the arsenic excreted in the bile reabsorbed in the intestine? Though it is known that organic arsenicals are poorly absorbed from the intestine, the form in which arsphenamines appear in the bile has not been determined. The bile from two dogs was collected for 24 hours following injections of 300 mg. neoarsphenamine. This bile contained 25 and 30 mg. of arsenic, respectively, and was returned to the duodenum of two other bile fistula dogs which had never before received arsenic. No arsenic was recovered from the bile of the second pair of dogs. Thus the biliary arsenic after injecting neoarsphenamine was not absorbed from the intestine.

Does arsenic accumulate in the bile after repeated weekly injections of neoarsphenamine or mapharsen? When 300 mg. of neoarsphenamine or 60 mg. of mapharsen was injected weekly for 5 to 8 weeks, the bile was repeatedly free of arsenic after seven days and no evidence of delayed excretion of accumulation of arsenic in the bile was observed.

DISCUSSION

The excretion of arsenicals by the liver of the dog: The results of this study confirm by a more direct method the conclusion of other investigators[14-17] that the liver is the predominant organ concerned in the excretion of arsenicals, and that the excreted arsenic does not undergo an enterohepatic circulation or absorption from the intestine. Within two or three days after injection, from 30 to 60 per cent, or an average of about 40 per cent, of the arsenic administered as neoarsphenamine or mapharsen in well-tolerated doses is excreted in the bile. It appears that mapharsen-arsenic is more rapidly excreted than neoarsphenamine arsenic. This may not be true, since 60 mg. of arsenic was given as neoarsphenamine while 17.4 mg. of arsenic was available for excretion after injecting mapharsen. It is of interest to observe that mapharsen may be more readily excreted, since it has been suggested that neoarsphenamine is converted to arsenoxide, or mapharsen, before it becomes actively treponemicidal in the body.

The process concerned in the excretion of mapharsen and neoarsphenamine is not one of simple diffusion. This is shown by the relatively slow excretion and by the failure of a choleresis to increase significantly the rate of arsenic excretion. These arsenical compounds, which are predominately removed from the blood by the liver, cannot be flushed out of the liver by producing a choleresis with bile acids.

It is evident that an increase in hepatic arterial blood flow does not facilitate the excretion of arsenicals. Sodium dehydrocholate, given intravenously or by mouth, increases hepatic arterial flow but does not increase the excretion of the arsenicals.

Do bile acids differ in their effect on the excretion of arsenical drugs? It has been clearly demonstrated that the liver handles conjugated unoxidized (glycocholic and taurocholic acids—Bilron, Lilly) and oxidized unconjugated (dehydrocholic acid—Ketochol and Decholin) bile acids differently.[3] When "ox-bile" acids are fed at least 90 per cent of them can be recovered in the bile as cholic acid. When dehydrocholic acid is fed or injected, only about 30 per cent can be recovered in the bile; the fate of the remainder is unknown. Probably, the oxidized bile acid is chemically changed and some of it may reach the peripheral circulation.

Our data show that glycocholic and taurocholic acids tend to increase and dehydrocholic acid tends to decrease the excretion of arsphenamine-arsenic in the bile.

One cannot conclude from the evidence in this paper that one type of bile acid would be more beneficial in protecting the liver from arsenical hepatitis than the other type of bile acid. The apparent decreased elimination of arsenic in the bile when dehydrocholic acid is given may not be due to retention of arsenic in the liver. On the contrary, the bile acid may pass into the peripheral circulation and increase capillary permeability to the arsenical and increase the amount of the arsenical in the general systemic tissues. If this is true, dehydrocholic acid would decrease the exposure of the liver to arsenic.

The results of giving dehydrocholic acid intravenously mixed with neoarsphenamine support the possibility that the bile acid allows the arsenical to pass more readily from the blood stream into tissues other than the liver only. Arsenic excretion in the bile was markedly decreased when the bile acid and neoarsphenamine were mixed and injected. When dehydrocholic acid was injected ten minutes prior to the arsenical injection, arsenic excretion was not decreased. Thus, when dehydrocholic acid is present in the circulation, simultaneously with neoarsphenamine, apparently less of the arsenical reaches the liver. Dehydrocholic acid is largely removed from the circulation in ten minutes after a single injection, and acts primarily upon the liver producing a choleresis, its effect on capillary permeability being transitory. This explanation is supported by the observations of Berman, Snapp and Ivy[5] on the effect of intravenously administered bile salts on the elimination of intravenously injected bilirubin. Bile salts injected with bilirubin decreased the amount of bilirubin excreted in the bile and increased the amount which entered the tissues, to such an extent that visible icterus occurred.

The effect of mixing sodium dehydrocholate with mapharsen on the excretion of arsenic in the bile was not studied because when the two are mixed a precipitate forms.

The "natural bile salts," sodium glyco- and taurocholate, were not used for intravenous injection in this study because they are more toxic[18] when given parenterally than sodium dehydrocholate.

Is it rational to give bile acids in an attempt to avoid arsenical hepatitis? If the observations on rabbits and on dogs hold true for man, the decreased amount of arsenic found in the liver and the amelioration of the toxic reactions observed by Appel and Jankelson[1, 2] when neoarsphenamine was administered with sodium dehydrocholate, is not due to greater excretion of the arsenical in

the bile. It is more likely due either to a greater dilution of the arsenical in the general body tissues, sparing the liver somewhat, or to a combination of the dehydrocholate with the arsenical so it becomes less toxic. The question will have to be answered by direct experiments in which the toxicity of the arsenical is compared when injected with and without dehydrocholic acid.

The accumulation of arsenic in the body in intensive syphilotherapy. It has been shown that nearly complete excretion of arsenic from a single injection of 60 mg. of mapharsen occurs within forty-eight hours in biliary fistula dogs, while neoarsphenamine-arsenic is largely excreted in seventy-two hours. In the five-day treatment of syphilis when the massive arsenotherapy method is used, mapharsen is given to the extent of 240 mg. in 24 hours for five days.[8-9] Thus, an injection of 60 mg. is repeated approximately every six hours. If arsenic is excreted in the human being as in the dog, considerable accumulation of arsenic will occur in five days, most of which would be in the liver.

According to observations made in this laboratory, the L. D. 60 of mapharsen or the dose required to kill 60 per cent of the animals is the same for dogs as for rabbits, namely, 13 mg. per kilo. In the rabbit, the results of Eagle and Hogan[19] indicate that a sublethal dose of mapharsen may be injected weekly without accumulative effect. Our results on the dog confirm their observation on the rabbit.

SUMMARY AND CONCLUSIONS

1. The excretion of neoarsphenamine and mapharsen-arsenic in the bile of dogs with a permanent bile fistula has been studied under standard conditions with and without the oral administration of a mixture of sodium glycocholate and taurocholate (ox-bile salts) and sodium dehydrocholate. The dose of neoarsphenamine was 300 mg. or 60 mg. of arsenic, and the dose of mapharsen was 60 mg., or 17.4 mg. of arsenic. The dose of arsenic was not given oftener than once a week and the animals weighed from 8 to 12, or an average of 10 kilograms.

2. In the case of neoarsphenamine, an average of 40 per cent of the arsenic administered was recovered in the bile in seventy-two hours. In the case of mapharsen, an average of 40 per cent of the arsenic administered was recovered in the bile in forty-eight hours. In both cases most of the arsenic is excreted in the bile during the first twenty-four-hour period and only traces are present in the bile after forty-eight to seventy-two hours. The results indicated that neoarsphenamine-arsenic is excreted more slowly by the liver than mapharsen-arsenic. It was shown that the arsenic excreted in the bile is not absorbed from the intestine.

3. If neoarsphenamine or mapharsen-arsenic is excreted by the human liver as by the canine liver, it is reasonable to assume that an appreciable accumulation of arsenic occurs in the liver when, for example, 240 mg. of mapharsen is administered daily for five days. However, in confirmation of Eagle and Hogan[19] and Magnuson and Raulston[14] who used rabbits, a sublethal dose may be given weekly without accumulation of arsenic in the liver.

4. The production of a choleresis *per se* in the dog does not augment the rate of excretion of neoarsphenamine or mapharsen-arsenic in the bile, because the oral administration of sodium dehydrocholate, which causes a brisk choleresis,

tends to decrease the elimination of the arsenic, and sodium glycocholate and taurocholate, which cause only a moderate choleresis, tends to increase the elimination of the arsenic in the bile.

5. The simultaneous intravenous administration of sodium dehydrocholate with neoarsphenamine decreases the rate of excretion of the arsenic in the bile, though a choleresis occurs. This result is probably due to the increased passage of the arsenical into the general body tissues secondary to the effect of the bile salts on capillary permeability. If the administration of dehydrocholic acid diminishes the hepatotoxic action of neoarsphenamine and mapharsen,[1, 2] it does not do so by increasing their excretion in the bile.

6. The results of this investigation do not provide a clear and substantial rationale for the administration of bile acids with arsenicals.

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LABORATORY METHODS

GENERAL

APPLICATION OF THE "INDUCTOGRAPH" TO THE REGISTRATION OF MOVEMENTS, PARTICULARLY OF BODY STRUCTURES SUCH AS THE PYLORIC SPHINCTER

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THE fact that an inductorium produces, respectively, more or less current as the secondary coil approaches or recedes from the primary coil has been extensively employed in biology (chiefly for the production of induced current to stimulate tissue). The same principle can be adapted to produce an "induction diagraph" or "inductograph" to be employed in the registration of changing positions of body structures, e.g., the movements of the heart or blood vessels, the various portions of the digestive tube, the bladder, ureters, uterus, skeletal muscles, etc. For such studies two suitable electromagnetic coils (a primary and a secondary coil) are fastened at opposite sides of the tissue whose movement is being studied and as the tissue contracts or relaxes, the coils will approach or recede, and correspondingly more or less current will be induced in the secondary coil. The inductograph is well suited for use in chronic experiments on unanesthetized subjects using imbedded coils or coils fastened to external surfaces of the body. Also, for special purposes it appears to offer advantages over apparatus commonly employed in acute experiments on anesthetized animals.

In this laboratory, the inductograph has been extensively employed for a year and a half in studying gastrointestinal movements. The "pyloric inductograph" has been used in 32 dogs for the chronic study of the pyloric sphincter movements under what appears to be rather physiologic conditions. In these animals, the two electromagnetic coils described below were placed with aseptic precautions so the coil cores and the sphincter diameter were in the same line. That is, the coils were sutured diametrically opposite to each other to the outer surface of the pyloric sphincter surface at the points of attachment of the lesser omentum and the greater omentum, respectively (Fig. 1). The lead wires of the coils were sutured along the lesser and greater curvature of the pyloric antrum and finally were brought to the outside through a stab wound. The coils and the lead wires were both covered with omental tissue. Coils examined after having been in place for several months were found to be firmly anchored by this omental tissue.

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The coils (Fig. 3) usually employed for the study of pyloric sphincter motility consist of a cross-shaped base of tinned brass 0.25 mm. thick. The three short arms were 5 mm. long, 1 mm. wide and each was pierced at the outer end with a 0.38 mm. hole to be used in suturing the coil in the location desired. The fourth arm was 10 mm. long, 3 mm. wide and was divided into two arms by a slot 1 mm. wide extending into the long arm for 7 mm. The arms were shaped to conform to the surface to which they were to be fastened. The core was a piece of tinned soft iron 1.25 mm. in diameter and 8.5 mm. in length. It was soldered to the base plate in such a manner as to form a 2.5 mm. projecting nib. The coil windings are insulated from the base plate by a thin fiber disk, and from the core by a layer of insulating lacquer.

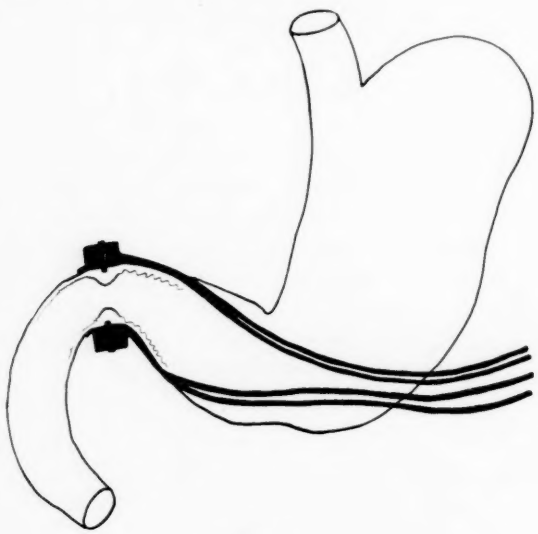

Fig. 1.

The coil proper consisted of approximately 1,000 turns of No. 40 Formex coated copper wire and was 6.25 mm. in diameter and 4.5 mm. thick. The lead wires were made by braiding together 10 strands of No. 40 Formex coated copper wire and three strands of No. 36 stainless steel suture wire. This was encased in $\frac{1}{16}$ x $\frac{1}{32}$ inch rubber tubing. One end of each rubber tube was slipped over a long arm of the base and secured with silk thread or with lacquer. The lead wires had previously been insulated from the base arm by a bushing made from a short piece of fine French woven catheter. The entire coil and base were coated with several layers of clear Polymerin No. 55* baked at 135° C. for one-half hour, or Bakelite lacquer XV-14633† baked at 135° C. for one hour.

The resistance of each coil was approximately 50 ohms. The inductive resistance offered by a coil to a 60 cycle alternating potential was small and thus the impedance of the coil under operating conditions was also approximately 50 ohms.

When the inductograph was in use, one coil (C_1, Fig. 2) was made the primary and was energized by connection in series with a suitable variable

*Courtesy of Ault and Wiborg Corportion, Cincinnati, Ohio.
†Courtesy of Bakelite Corporation, Bloomfield, N. J.

resistance (R) to the 1.5 volt tap of a 60 cycle filament transformer (T). The oscillating magnetic field generated by C_1 induced in the secondary coil (C_2) a small alternating potential which in turn was amplified by a radiotronic amplifier (A) having a rated output of 13 watts and a maximum gain of 112 decibels. The amplifier output was arranged to energize the mirror oscillograph (M) of the Victor type having an impedance of 5000 ohms. A beam of light from the

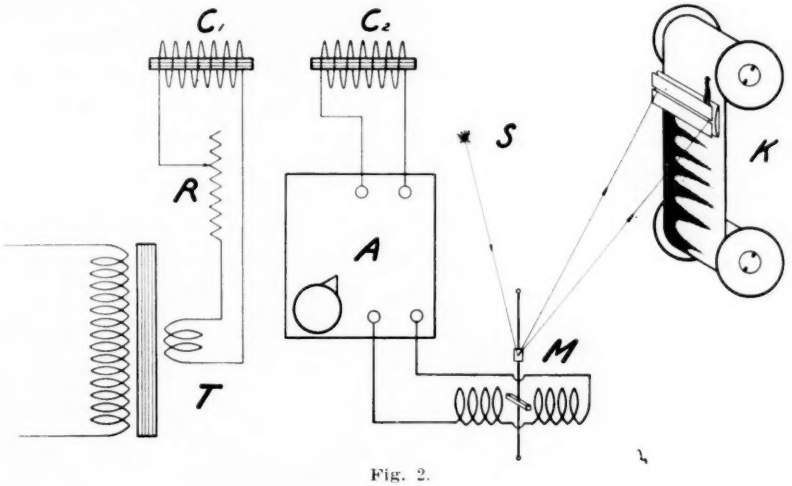

Fig. 2.

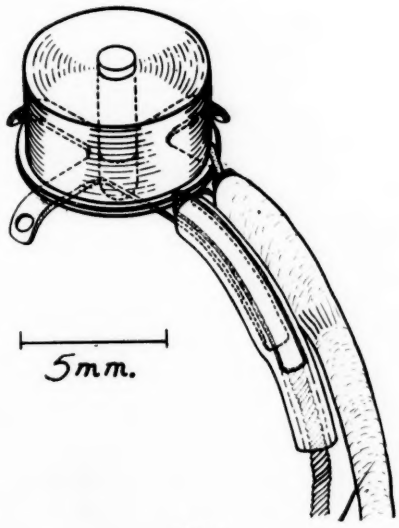

Fig. 3.

slit lamp (S) was reflected from the oscillograph mirror onto the sensitized paper of a photokymograph (K). Since the impressed current was oscillating, the oscillograph produced an oscillating band of light which lengthened as the coils approached each other and shortened as they separated. We customarily recorded only the upper portion of the band and thus obtained a record, the peaks of which indicated approximation of the coils (interpreted as pyloric contraction), while the troughs indicated separation of the coils (pyloric relaxation or opening).

The significance of several possible errors and artifacts was investigated as follows:

1. The passage of peristaltic waves over the pyloric region may rotate the coils about the lateral axis (rocking on the coil base). Table I details the effects obtained in vitro at various interpolar distances by rotating one coil about its base and shows that moderate angular rotation has an essentially negligible influence on the magnitude of the e.m.f. induced in the secondary coil. Furthermore, fluoroscopic observations of animals having implanted coils show that peristaltic contractions and other normal phenomena rotate the coils less than 5° from the longitudinal axis. Thus, the possibility of error due to coil rotation is negligible.

TABLE I

EFFECT OF ROTATING ONE COIL ABOUT LATERAL AXIS

ANGLE	BAND LENGTH WITH INTERPOLAR DISTANCE OF: 7.5 MM.	14 MM.	19 MM.
0°	94 mm.	19.5 mm.	16.5 mm.
5°	94	18	16.5
10°	94	18	16.5
15°	91	18	16.5
20°	91	18	16
25°	89	17.5	16
30°	89	17.5	15

2. Increasing the intrapolar distance by lateral displacement of the coils is about as effective as longitudinal displacement in modifying the band length (curve *XF*, Fig. 4). Fluoroscopic examinations of implanted coils at the pyloric sphincter fortunately indicate that in practice, significant lateral displacement does not occur.

3. Placing soft tissue, bone, wood, glass, water, normal saline, barium sulfate, etc., between or around the coils of an inductograph did not modify the magnitude of e.m.f. induced in the secondary coil. Probably this was related to the low ferro-magnetic content of these substances.

4. When the magnitude of current ordinarily employed under experimental conditions was allowed to pass through the primary coil for 30 minutes, no perceptible elevation of coil temperature occurred. Thus, the heat developed in the primary coil probably did not injure the tissue in which it was imbedded or modify its activity.

5. No detectable e.m.f. was induced in the leads of the secondary coil by passing a current of the magnitude ordinarily used in the primary circuit through "dummy" leads placed adjacent to the secondary leads.

6. Cutting of lines of flux by the motion of the coils themselves might induce current. Ordinary sphincter peristalsis, however, would only move the coils approximately 0.5 cm. in 5 to 7 seconds and this interval is too long in comparison to the time required for the primary coil to vary the flux density between zero and its maximum value ($\frac{1}{240}$ second) to induce significant current in the secondary coil. We found that normal peristaltic movements were too slow to induce detectable e.m.f. in the secondary coil by cutting lines of force even when the primary was energized by direct current.

A series of typical calibration curves made in vitro are shown in Fig. 4. They belong to the family of hyperbolic curves having an exponential factor somewhat greater than 2. Each curve has a steep, approximately linear portion and this section of the curve has been used in our experiments.

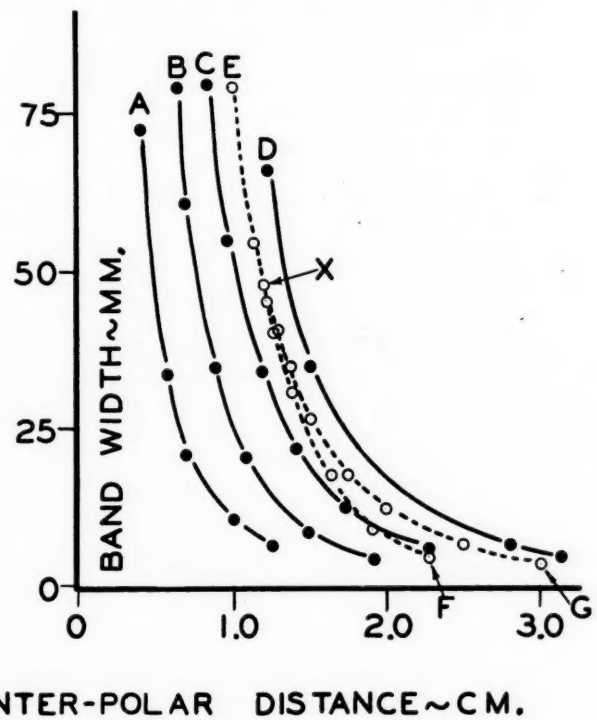

Fig. 4.—In vitro calibration curves of a pair of coils used in a pyloric inductograph showing effects on the band width of *longitudinal* displacement of the coils when employing a gain control setting of A-5, B-10, C-15, D-20. With a different pair of coils and a gain control setting of 15, the effect of longitudinal displacement is shown in E X G. XF shows the effect of increasing the interpolar distance by *lateral* displacement of one coil from an original interpolar position at X of 1.2 cm.

The characteristic motility of the pyloric sphincter as recorded by the pyloric inductograph consists of rhythmic contractions having a 10.4-14.1 second cycle. In the fed animal, they tend to be more uniform, more continuous, and of greater magnitude than during periods of fasting. A detailed description of this activity and its relation to gastric evacuation will be given elsewhere.

NEW SIMPLE SILVER STAIN FOR DEMONSTRATION OF BACTERIA, SPIROCHETES, AND FUNGI IN SECTIONS FROM PARAFFIN-EMBEDDED TISSUE BLOCKS

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IN 1937 a simple method for the demonstration of spirochetes in frozen sections was published.[1] One of the disadvantages of this method was that in steps 8 and 13 a strong heat to the bubbling point had to be used and the presence of the technician was consequently required. A second disadvantage was the use of frozen sections. For these two reasons a silver staining technique for organisms in routine paraffin sections was sought for. The new method given below has been used for the last three years. Sections from the formalin-fixed tissue blocks embedded in paraffin were cut 6 to 7 microns thick and mounted on slides with clean egg albumen. *Zenker-fixed tissue could not be used,* nor could celloidin films be used.

METHOD OF STAINING

1. Deparaffinize sections in the usual manner.

2. Place sections in an aqueous 1 per cent uranium nitrate solution for 3 minutes.

3. Wash twice in distilled water.

4. Immerse in a 1 per cent aqueous solution of silver nitrate (e.p.) for 2 hours in the oven (56 to 58 degrees).

5. Wash thoroughly in distilled water.

6. Dehydrate up to absolute alcohol and place sections in 2.5 per cent alcoholic (absolute) gum mastic solution for 5 minutes.

7. Without washing them transfer sections directly from the gum mastic solution into the reducing solution of hydroquinone (1 Gm. hydroquinone dissolved in 60 c.c. distilled water or equivalents, to which are added 20 c.c. of a 2.5 per cent alcoholic [absolute] gum mastic solution and 20 c.c. of silver nitrate sodium potassium tartrate solution, see below). Mix these three solutions thoroughly by moving the track with the slides up and down for some seconds in the dish. Leave the slides in the reduction mixture for twelve to fifteen minutes until the color of the sections changes to light brown.

8. Wash three times in distilled water, dehydrate, clear in xylol, and mount in Canada balsam.

CHEMICALS

Uranium nitrate, e.p., Baker's analyzed.
Silver nitrate, e.p., Baker's analyzed.
Gum mastic, powderized, Eimer and Amend.

From the Brain Disease Registry, Department of Pathology, Wayne University College of Medicine, Detroit, Michigan.
Received for publication, Mar. 20, 1944.

Hydroquinone, Ceneo.
Sodium potassium tartrate powder, c.p.
Xylene, c.p., Baker's analyzed.
Distilled water or, preferably, twice distilled water for steps 3 and 5 and for preparing the silver nitrate solutions.

SOLUTIONS

A. Gum Mastic Solution.—The solution is made by adding 2.5 Gm. of powderized gum mastic to 100 c.c. of absolute alcohol. It is dissolved by stirring with a glass rod and is left standing overnight. The solution is filtered several times until clear.

B. Reduction Mixture.—The silver nitrate sodium potassium tartrate solution is prepared as follows: 2 Gm. of silver nitrate (c.p.) are dissolved in 1,000 c.c. of boiling distilled water. When the chemical is dissolved and the water is boiling, 1.65 Gm. of powdered sodium potassium tartrate is quickly added until the white precipitate changes to gray. Then the solution is filtered while hot into an amber bottle with glass stopper. The silver nitrate sodium potassium tartrate solution is poured into the alcoholic gum mastic solution and not vice-versa; equal parts, 20 c.c. each, of silver nitrate sodium potassium tartrate solution and of 2.5 per cent alcoholic gum mastic solution are used. A milky fluid results which is the 40 c.c. added to the dish containing the 60 c.c. of hydroquinone solution (1 Gm. of hydroquinone dissolved in 60 c.c. of twice distilled water) and the slides.

The silver nitrate sodium potassium tartrate solution is the same as that used in the silver mirror method for smears.[2]

The final concentrations in the reduction mixture are

hydroquinone 1 per cent,
gum mastic 0.5 per cent, and
silver nitrate less than 0.04 per cent,

because the precipitate formed in the sodium potassium tartrate solution has been eliminated by filtration.

The 2.5 per cent alcoholic gum mastic solution, the 1 per cent aqueous uranium nitrate solution, the 1 per cent aqueous silver nitrate solution, and the silver nitrate sodium potassium tartrate solution can be kept indefinitely in stock, the last two in amber bottles with glass stoppers in the refrigerator. The hydroquinone solution has to be prepared each time shortly before use.

Instead of staining dishes with removable racks (20 slides may be accommodated if the slides are placed back to back in pairs), Coplin jars can be used which accommodate 8 slides, three pairs in the inner grooves and one slide in each outer groove.

In using Coplin jars care has to be taken that the concentrations of the solutions are equivalent to those previously given; for example, hydroquinone solution has to be made up by dissolving 0.5 Gm. in 30 c.c. of distilled water; consequently 10 c.c. of 2.5 per cent alcoholic gum mastic solution and 10 c.c. of silver nitrate sodium potassium tartrate solution are used. In our laboratory, staining dishes of 200 c.c. capacity were used so that 2 Gm. of hydroquinone were dissolved in 120 c.c. of distilled water; 40 c.c. of 2.5 per cent alcoholic gum

mastic solution and 40 c.c. of silver nitrate sodium potassium tartrate solution were mixed together and added to the 120 c.c. of hydroquinone solution.

Glass rods are used to handle the transfers of the racks with the slides from one staining dish to the other. All glassware must be clean and rinsed in distilled water previous to use. The staining dish which contains the gum mastic solution should be rinsed with absolute alcohol previous to use. The dishes with the dehydrating solutions should be freshly prepared and not used more than once. In using Coplin jars the upper corner of the slide should not be immersed into the solution so that the slides can be removed and put into the next Coplin jar without touching the solutions with forceps or any other metallic instrument.

The time required for the preparation of sections is less than 2½ hours; 2 hours of this period can be utilized by the technician for other work, since the slides are put into the oven and left there for this length of time (step 4). Comparison with other methods shows that the time required to finish the sections is shorter than in Dieterle's method.[3] Furthermore, in staining spirochetes Dieterle not alone uses gum mastic, introduced by me in 1922,[4] but he uses pyridine, sodium sulfite, acetone, and formaldehyde, four chemicals not necessary in our new method. Krajian[5] has published two methods for staining *Spirochaeta pallida* in single frozen sections. Both methods, the first one comprising sixteen steps and the second one eighteen steps, require a double impregnation of sections with silver nitrate (steps 7 and 11 and 7 and 12, respectively). In Krajian's first method the uranium nitrate mixture and the solution of silver nitrate have to be warmed to 67° C. before sections are placed in them. With the sections in them the uranium nitrate mixture requires 15 minutes to be heated to 67° C. and the solution of silver nitrate about 30 minutes. Krajian's second, his so-called twenty-minute staining method requires the continuous presence of a technician because in step 8 the silver nitrate solution has to be heated over a Bunsen burner until bubbles form and the temperature has then to be kept at 70° C., the solution being heated with the burner every few seconds for 7 minutes. Furthermore, Krajian's methods not only require uranium nitrate, gum mastic, silver nitrate, distilled water, absolute or 95 per cent alcohol, and hydroquinone, but in addition formic acid, formaldehyde, glycerine, acetone, sodium sulfite, and pyridine, six chemicals not used in our new method. Even though sodium potassium tartrate is used by us and not by Dieterle and Krajian, we still use three to five chemicals less than do the above investigators. Hence, compared with the methods mentioned above, the new method described is much simpler and cheaper, and it is applicable to routine paraffin sections.

By the new method bacteria, spirochetes, and fungi present themselves with a black mirrorlike coat and are easily distinguished from the yellow or brown tissue elements. The color contrast is very deep, facilitating the search for rare organisms. By this new method *Treponemata pallida* were easily demonstrable in primary skin lesions, in secondary syphilis of the skin, in a primary chancre of the uterine cervix, in organs affected by congenital syphilis,[6] in the brains of general paretics, and in experimental syphilis of rabbits.[7] Tissues with other spirochetes were also stained: human kidneys in Weil's disease, kid-

neys of rats showing *Leptospira icterohemorrhagiae,* and tonsils containing oral spirochetes. Other organisms stained in the tissues by this new method were pneumococcus in cases of purulent meningitis, meningococcus in cases of meningococcic meningitis, *Bacillus welchii, Mycobacterium tuberculosis* in lung, *Mycobacterium leprae* in skin lesions of leprosy, *Torula histolytica* in three cases of meningoencephalitis due to *Torula histolytica,*[8] *Actinomyces asteroides* in a brain abscess, *Aspergillus mycelia* in a case of aspergillosis with multiple abscesses in the brain, and *Histoplasma capsulatum* in a case of human histoplasmosis. It is interesting to note that protozoa do not take the silver coating in the form of the black shiny metallic phase; for example, the metallic phase was not seen covering the toxoplasmas in a case of toxoplasmic encephalitis nor in the organs of rats infected with *Trypanosoma lewisii.*

The sections do not show artifacts; particularly, there are no precipitations of metallic silver. Melanin granules, calcified material, and the so-called formalin pigment show a higher affinity to the metallic silver phase than other tissue elements; however, these structural elements are easily distinguished from the organisms by their location and morphological appearance.

SUMMARY

A simple new silver stain for the demonstration of bacteria, spirochetes, and fungi in sections from paraffin-embedded tissue blocks is reported and compared with other methods. This method has been used for the last three years without failure.

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A HOMEMADE MECHANICAL PIPETTE

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AN AUTOMATIC pipette is almost indispensable in a laboratory handling large numbers of Kahn and Wassermann tests. When the serological service was instituted in the Canadian Army Overseas, it was found impossible to produce an automatic or a mechanical pipette, and the problem arose of making one from the material at hand. The following is a description of an automatic pipette designed and built by the writer, and in use for the past year. This machine is easy to handle and delivers accurately amounts from 0.25 c.c. up.

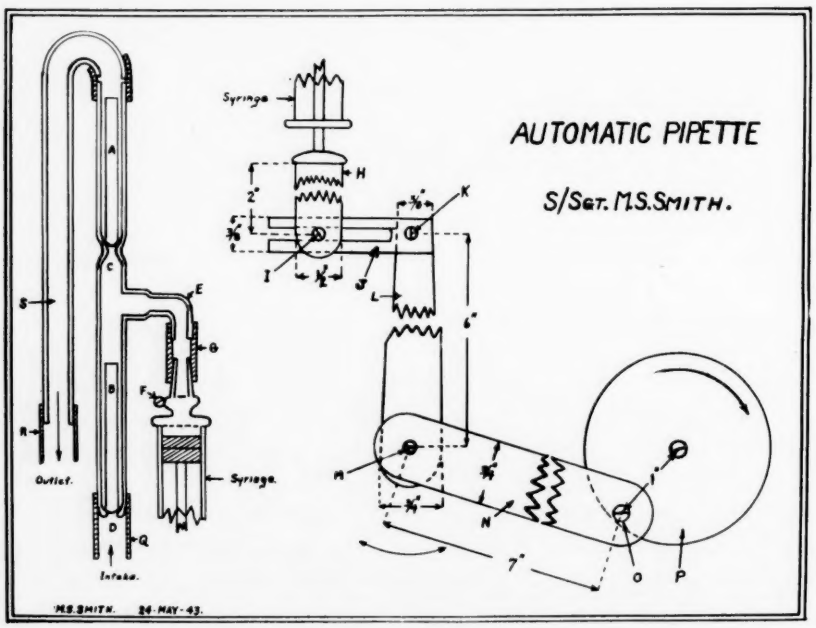

Fig. 1.

The motor is the type used for the Van Slyke apparatus, is geared, and has a variable speed. In the case of this machine, it was necessary to use electric light bulbs in series to cut down the voltage from 220 v to 110 v. The flywheel *P* has an eccentric and is attached by a series of lever arms to the plunger of a 2 c.c. Record syringe. The syringe may be adjusted for amounts from 0.25 c.c. up by moving the bearing *I* along the slot in *J*. The flow through the pipette is directed by valves *A* and *B*. On the downstroke of the plunger, *A* closes and *B* opens; on the upstroke the reverse occurs, ensuring a regular even flow of fluid along *S* to rubber pressure tubing and thence to test tubes. Details of the

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machine may be better understood by reference to Fig. 1. The flywheel *P* moves the syringe plunger through the medium of the members *T*, *L*, and *N* which are made of strap iron and have bearings at *O*, *M*, *K*, and *I*. The syringe

Fig. 2.

Fig. 3.

nozzle is hinged at *F* by a piece of brass rod $\frac{1}{8} \times 1\frac{1}{2}$ inches soldered to it. At *G* is a piece of rubber pressure tubing connecting the syringe to the *T* tube and valve system. In the valve system a good grade glass *T* tube $\frac{3}{16}$ inch inside

diameter is used. One end is partially closed in a flame for valve seat *D*. Valve *B* is made first, before the tube is drawn out for valve seat *C*. To make the valves, take a piece of glass rod about 12 inches long that will have about 1/16 inch clearance when inserted into the *T* tube. Smooth one end in a flame and insert into the T tube for a trial fit. The valve and valve seat are ground to fit with pumice and turpentine, using a rotating motion. When a fit is obtained, the rod is then cut about 1½ inches from the ground end and this shorter piece constitutes the valve *B*. After valve *B* is in place, the other end of the *T* tube is carefully drawn out in a flame for the valve seat *C*, and valve *A* is ground to fit in the same way as valve *B*. The stem *E* of the *T* tube is then bent downwards to connect with the syringe system by means of rubber pressure tubing at *G*.

The motor is covered with a plain box for protection and to give support for bearing *K* and to the valve assembly (Fig. 2). The whole apparatus is constructed from material at hand and is a very useful efficient machine (Fig. 3).

CHEMICAL

DETERMINATION OF TOTAL AND FREE CHOLESTEROL

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NUMEROUS methods are available for the determination of total cholesterol, most of them based on the Liebermann-Burchard reaction, in which a blue color develops when cholesterol is treated with acetic anhydride and concentrated sulfuric acid. The majority of these tests are concerned with various steps of extraction of the cholesterol from the blood.

The color produced in the Liebermann-Burchard reaction is evasive and of short duration. The complete intensity is attained in a few minutes, depending on temperature and light factors. The peak of the color intensity lasts but a few minutes and then fades rather rapidly. It is essential, therefore, to take colorimetric readings of the unknown and the standard at the same time. When several determinations have to be made, the later colorimetric readings may be outside the peak of the curve, giving an error in results, especially when a permanent standard is used, or a permanent curve is plotted with a photoelectric colorimeter.

The method herein described produces a more stable color. By adding glacial acetic acid to the Liebermann-Burchard reagents, the color develops much more slowly, and the peak of color intensity is of much longer duration, very gradually fading away. Chart 1 is a graphic presentation of the color development with and without the addition of glacial acetic acid, as recorded by a photoelectric colorimeter (Klett), under identical conditions of temperature and light. The heavy line is a record of results with the use of glacial acetic acid; the light line, without. The abscissa represents the time and the ordinate, the colorimetric readings. It can be seen from the chart that without glacial acetic acid the color develops rapidly, reaching its maximum intensity in nine minutes. The peak of the color intensity remains for five minutes, then rapidly fades. Twenty minutes after reaching its maximum intensity, the color has faded 18 per cent. With the addition of glacial acetic acid, however, the color reaches its maximum intensity in twenty-one minutes, remains at a peak for fourteen minutes, then begins to fade much more slowly. Twenty minutes after reaching its maximum intensity, the color has faded only 1 per cent. The color produced with glacial acetic acid, although slightly less intense at its peak than the color produced without glacial acetic acid, nevertheless is sufficiently deep to make accurate determinations even with small amounts of cholesterol present.

Several experiments were made with various stocks of glacial acetic acid as follows: (1) a new bottle; (2) a bottle that had been kept open for several weeks; (3) glacial acetic acid to which 1 per cent water was added. Invariably, all colorimetric readings were the same.

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EXTRACTION OF THE CHOLESTEROL

Various methods were studied for the extraction of cholesterol from the blood, and it was found that the most thorough and expedient method which did not require special apparatus was to extract the lipids from the blood by means of acetone-alcohol mixture, evaporate the extract, and then isolate the cholesterol from the residue with chloroform.

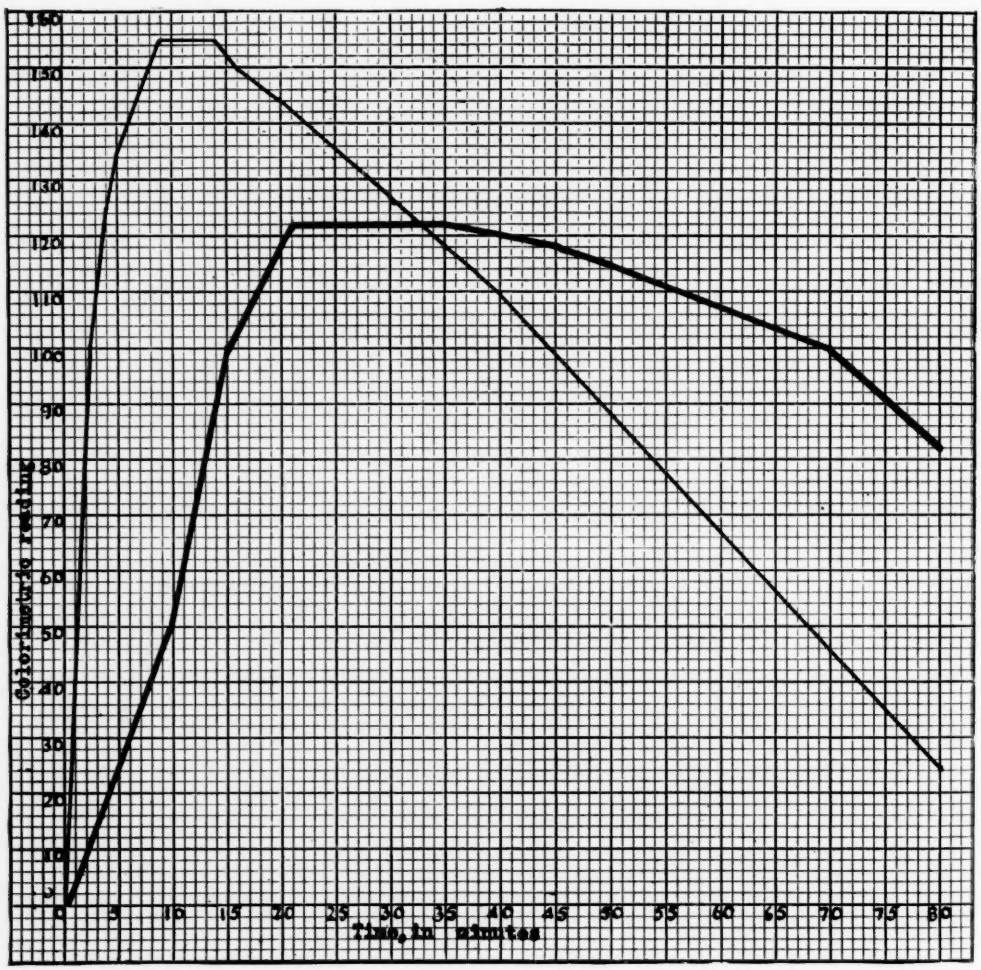

Chart 1.—Color development with and without glacial acetic acid. Heavy line with glacial acetic acid, light line without.

PROCEDURE FOR TOTAL CHOLESTEROL

Reagents.—

1. Acetone-alcohol, equal parts of acetone and absolute alcohol.
2. Chloroform, C.P., reagent.
3. Glacial acetic acid.
4. Acetic anhydride.
5. Concentrate sulfuric acid.

6. a. Stock cholesterol solution, containing 1 mg. of cholesterol in 1 c.c. of chloroform.
   b. Working standard: dilute 1 c.c. of the stock to 10 c.c. volume with chloroform.

Technique.—

Introduce into a Pyrex test tube graduated at 5 c.c., or into a graduated centrifuge tube, 4 c.c. of acetone-alcohol mixture. Add to it slowly, 0.5 c.c. of oxalated blood. When determination of free cholesterol is also required, use a test tube graduated at 10 c.c.; add to it 8 c.c. of acetone-alcohol and 1 c.c. of oxalated blood. Immerse test tube in hot water of a temperature near boiling point until the solution in the test tube begins to boil. Then remove the test tube from the water bath and stir contents thoroughly with a stirring rod. Repeat this boiling process twice more. Let stand two or three minutes, and then add acetone-alcohol mixture to the 5 c.c. mark (10 c.c. mark for free cholesterol). Stir thoroughly. Insert a cork into test tube, and centrifuge for about three minutes at moderate speed. Decant supernatant fluid into a clean test tube. Use 3 c.c. of this extract for the total cholesterol; 4 c.c. for free cholesterol. Where total cholesterol alone is required, 3 c.c. can easily be pipetted off without transferring into another test tube. Transfer 3 c.c. of the extract into a 100 c.c. dry Pyrex beaker and evaporate slowly, just to dryness. To prevent too rapid evaporation, it is advisable to place a piece of asbestos on top of the electric plate. After the residue in beaker is dry, add to it about 5 c.c. of chloroform and evaporate slowly to about half volume. Transfer the extract to a dry test tube graduated at 5 c.c. Repeat extraction process until the 5 c.c. mark is reached. Let stand for a few minutes to cool; then add 1 c.c. of glacial acetic acid, 2 c.c. of acetic anhydride, and 0.2 c.c. of concentrated sulfuric acid. Insert rubber stopper, invert twice, and place tube immediately into a water bath of 23° C. for twenty-five minutes. Examine in colorimeter. A red filter facilitates matching of colors. In order not to have excessive variations in light, it is advisable to develop the color in the same corner of the laboratory. We have found that a brown glass jar is very useful for such a water bath. Prepare the standard by adding 5 c.c. of the working standard, 1 c.c. of glacial acetic acid, 2 c.c. of acetic anhydride, 0.2 c.c. of concentrated sulfuric aid; treat this mixture in the same way as the unknown. This standard represents 167 mg. cholesterol per 100 c.c.

TABLE I

NUMBER OF TEST	PRESENT METHOD TOTAL CHOLESTEROL IN MG. PER 100 C.C.	BLOOR METHOD TOTAL CHOLESTEROL IN MG. PER 100 C.C.
1	162	158
2	213	214
3	186	192
4	204	202
5	354	364
6	180	184
7	421	417
8	167	167
9	210	212
10	208	206
11	196	198
12	210	206

Table I shows comparative results obtained with the method herein described and the Bloor method. In the latter only two tests were run at the same time, and a fresh standard was made for each determination. The color of the unknown and the standards were developed under identical conditions of temperature and light. The extraction procedure in this method does not produce any extraneous color, since there is a tendency in this method to give slightly lower values, as compared with other methods employing the alcohol-ether extraction.

METHOD FOR FREE CHOLESTEROL

Reagents.—

Above reagents as employed in method for Total Cholesterol, plus:

1. Digitonin solution: 1 per cent digitonin in 95 per cent alcohol.
2. Ether-acetone mixture, 2 parts to 1.
3. Standard solution containing 0.5 mg. cholesterol in 1 c.c. glacial acetic acid.

Technique.—

To 4 c.c. of acetone-alcohol extract, add 1 c.c. of digitonin solution and 1 c.c. of distilled water. Mix, and allow to stand for about forty minutes. Centrifuge for ten minutes at high speed. Decant supernatant fluid and let the inverted test tube stand over a piece of filter paper for two to three minutes. Wipe mouth of test tube with filter paper, and add 10 c.c. of ether-acetone mixture, washing sides of the test tube well with this mixture. Stir precipitate and centrifuge for ten minutes at high speed. Decant supernatant fluid, drain again as before, and add again 10 c.c. of the ether-acetone mixture. Stir precipitate and centrifuge for ten minutes at high speed. Decant supernatant fluid, dry mouth of test tube with filter paper and immerse test tube in warm water at 50° C. for a few minutes (to dry precipitate). Now add 3 c.c. of glacial acetic acid, immerse in water heated to 70° or 80° C. for a few minutes, or until the precipitate is completely dissolved. Cool the test tube. Now add 2 c.c. of acetic anhydride, 0.2 c.c. of concentrated sulfuric acid, stir thoroughly, and allow to stand in a water bath at 23° C. for 1½ hours. Examine in colorimeter.

PREPARATION OF STANDARD

To 1 c.c. of standard solution containing 0.5 mg. of cholesterol, add 2 c.c. of glacial acetic acid, 2 c.c. of acetic anhydride, and 0.2 c.c. of concentrated sulfuric acid. Stir thoroughly and allow to stand for 1½ hours in a water bath of 23° C. This standard represents 125 mg. of free cholesterol. To determine cholesterol esters, deduct free cholesterol from the total cholesterol.

SUMMARY

Blood is extracted with acetone-alcohol 1:1, the extract evaporated and extracted with chloroform. Glacial acetic acid is added to the chloroform extract. The addition of the glacial acetic acid produces a more stable color of Liebermann-Burchard reaction. The acetone-alcohol extract is also being used for the determination of free cholesterol.

SULFONAMIDE DETERMINATION IN BLOOD USING XYLENESULFONIC ACID AS A PROTEIN PRECIPITANT

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SULFONAMIDES are ordinarily determined in blood according to the method of Bratton and Marshall using trichloracetic acid as the protein precipitant. Because of the acute shortage of trichloracetic acid in the Southwest Pacific Area, a method has been devised in which the protein is precipitated by xylenesulfonic acid. The possibilities of this reagent were suggested by the use of p-toluenesulfonic acid in an older method of Marshall.[1] The synthesis of p-toluenesulfonic acid was precluded by the scarcity of toluene. Xylene, on the other hand, is readily available, as is technical sulfuric acid.

METHOD

Protein is precipitated from blood with a mixture of xylenesulfonic and sulfuric acids, and sulfonamides are determined by coupling in alcohol solution with N-(1-nephthyl)—ethylenediamine dihydrochloride according to a modified Bratton-Marshall method.[2]

REAGENTS

a. Xylenesulfonic acid. Place 30 c.c. xylene and 40 c.c. concentrated sulfuric acid (technical grade is satisfactory) in a 125 c.c. flask. Warm to start the reaction and shake at intervals until a nearly clear solution is obtained (about 30 minutes). Separate the small amount of unreacted xylene. Dilute to 250 c.c. with water. Bring to a boil in a 1,000 c.c. beaker. Cool, and add slowly, with stirring, a suspension of 34 Gm. calcium hydroxide in 50 c.c. water. Boil for one minute, and filter with suction. Wash thoroughly with three 10 c.c. portions of hot water, stirring up the mass each itme. Dilute the filtrate to 280 c.c. Allow to stand for two days and decant from the precipitant formed. This gives approximately a 20 per cent solution.

b. Sulfuric acid. N/12.

c. Xylenesulfonic-sulfuric acid mixture. To 15 volumes N/12 sulfuric acid (b) add 4 volumes xylenesulfonic acid (a).

d. Sodium nitrite 0.1 per cent. Freshly prepared.

e. Ethyl alcohol. 95 per cent.

f. N-(1-naphthyl)—ethylenediamine dihydrochloride, 0.1 per cent. Keep in refrigerator in brown bottle.

g. Stock standard sulfonamides. 100 mg. per liter for all except sulfadiazine (50 mg. per liter). Keep in refrigerator.

h. Strong working standard. 1 c.c. stock (2 c.c. for sulfadiazine) diluted to 20 c.c. with xylenesulfonic-sulfuric acid mixture (c).

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i. Weak working standard. 1 c.c. stock (2 c.c. for sulfadiazine) diluted to 50 c.c. with xylenesulfonic-sulfuric acid mixture (e).

PROCEDURE

Add 1 c.c. oxalated blood dropwise with shaking, to 15 c.c. N/12 sulfuric acid (b). Allow to lake for two minutes. Then add, dropwise with shaking, 4 c.c. xylenesulfonic acid (a). Filter. One cubic centimeter oxalated blood may be added alternatively to 19 c.c. xylenesulfonic-sulfuric acid mixture (e). To 10 c.c. filtrate and to 10 c.c. each of the strong and weak standards, add 0.5 c.c. sodium nitrite solution (d). Mix and allow to stand three minutes. Add 5.0 c.c. ethyl alcohol (e) and mix. Add 0.5 c.c. N-(1-naphthyl)-ethylenediamine dihydrochloride (f) with vigorous swirling. Allow to stand at least a minute and compare in the colorimeter, using the closer matching standard. The colors are stable for several hours.

CALCULATIONS

Weak standard:

$$S/U \times 4 = \text{mg. per 100 c.c. blood.}$$

Strong standard:

$$S/U \times 10 = \text{mg. per 100 c.c. blood.}$$

Recovery experiments and comparative studies were made employing both trichloracetic acid and xylenesulfonic acid as precipitating agents (Table I).

TABLE I

BLOOD LEVELS (IN MG. PER 100 C.C.) OF FREE SULFONAMIDES OBTAINED IN RECOVERY EXPERIMENTS AND COMPARATIVE STUDIES USING TRICHLORACETIC ACID AND XYLENESULFONIC ACID AS PRECIPITATING AGENTS

SULFONAMIDE	PREPARED CONCENTRATION	TRICHLORACETIC ACID METHOD	XYLENESULFONIC ACID METHOD
Sulfadiazine	10.0	9.5	9.6
	5.0	4.2	4.0
	----	5.9	5.7
	----	10.1	9.5
	----	4.3	4.2
Sulfathiazole	10.0	9.4	9.2
	10.0	--	9.1
	10.0	--	8.9
	10.0	--	9.4
	10.0	--	9.0
	10.0	--	9.4
	5.0	4.1	3.9
Sulfaguanidine	10.0	9.4	9.5
	5.0	4.1	4.2
	2.0	--	2.0

SUMMARY

An alternate method for determining blood sulfonamides has been described. The method offers a substitute protein precipitant, prepared from readily available laboratory reagents. Results are comparable with those obtained by the standard trichloracetic acid method.

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A MICRO-DECANTER FOR CENTRIFUGE WASHING

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IN SEVERAL procedures for microchemical analysis of metals such as calcium or potassium in biological material, it is necessary to separate the precipitate from the wash fluid by centrifugation. Some workers simply pour off the supernatant fluid by tilting the tube, while others draw it off through a glass tube with bent tip using power suction. Each method risks the loss of floating precipitate. In the second, the flakes of precipitate are swept up by the blast of air that enters the tip of the tube as it breaks through the surface

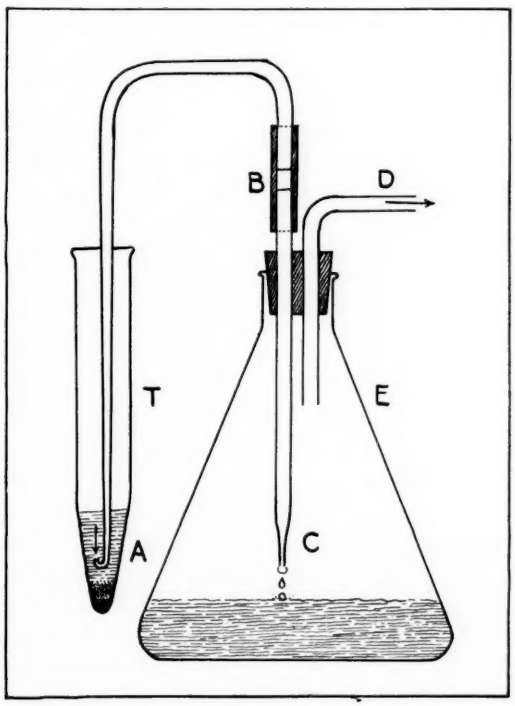

Fig. 1.

film when the decantation is stopped. The device presented avoids this difficulty by using a balanced siphon in which flow ceases just before its tip is pulled through the surface film. Fluid is removed to the last fraction of a drop, and if by accident any precipitate is sucked up, it can be returned since the flow is reversible.

Fig. 1 illustrates the design and operation of the decanter. A unique feature is the narrow tip *C* which prevents fluid from falling out of the siphon *ABC* and allows removal from the centrifuge tube *T* of the last small drop;

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another is the rubber joint *B* by which the arms of the siphon are adjusted in length until the fluid columns balance when *T* is removed. Before use the siphon is filled with wash fluid by suction at *D*, preferably by mouth, by way of a tube with mouthpiece.

With tube *T* in position the siphon flows; the flow may be accelerated by suction, stopped suddenly, or even reversed by pressure at *D*. Because of the balanced siphon, suction by mouth requires little effort and gives more control than is possible through any mechanical source. Just before the tip disengages the fluid, the suction is removed.

The decanter is best mounted by clamping the neck of flask *E* to a ring stand. The centrifuge tube *T* is held in place and manipulated mechanically by an adjustable screw or simply steadied by thumb and forefinger against a ring stand.

SUMMARY

A micro-decanter for the separation of the wash fluid from a metallic precipitate in centrifuge washing is described. Use of a balanced siphon rather than power suction gives delicate control so that accidental removal of floating flakes of the precipitate is avoided.

BOOK REVIEWS AND NOTICES

The Riddle of Cancer. By Charles Oberling, M.D., translated by William H. Woglom, M.D. Yale University Press, New Haven, Conn. Price $3.00. Cloth with 196 pages.

Aside from two introductory chapters this book of 196 pages is divided into three parts: first, an unbiased exposition of three theories of the nature of cancer; second, a discussion of modern experimental research in cancer; and third, a vigorous defense of the author's theory of the cause of cancer—that it is caused by viruses.

As would be expected and is natural, there is considerable tendency to emphasize the contributions of the French, to the exclusion (perhaps only nonmention) of others. The position of Virchow, Cohnheim, and others, however, is clearly made and the only charge is pardonable provincialism.

Whether or not the laity can derive instruction and information must remain for the laity to determine for themselves. A fair statement is on the paper jacket. "It is a book for laymen as well as doctors since it is written with a simplicity that does not flinch from using technical terms or defining them." There can be little question of the simplicity and logic of the discussions (which is as much to the credit of the translator as of the author). On the other hand every page is sprinkled with scientific terms, some of which are not defined. A guess might be that the educated lay person will find the style and context sufficiently intriguing to stimulate him to read. In sarcasm, the author is at his best. For example on page 34 of the section devoted to the microbic or parasitic hypothesis of cancer: "Such a spectacle was presented hardly eight years ago, when it was announced to an astonished world that tomatoes are the cause of cancer. Not only announced but proved! For the injection of tomato juice into the peritoneal cavities of rats was said to have been promptly followed by sarcomas, and photomicrographs were offered in support of the assertion. Well, it was difficult to incriminate such a common article of diet, so a microorganism was sought, and as though by magic one appeared: an inoffensive bacillus of the subtilis group that everyone carries in his skin. But there, it was said, were the proofs; this microbe, isolated from tomato juice, had been obtained in pure culture, and produced sarcoma when inoculated into rats. No one, of course, was able to confirm these experiments and both tomato and subtilis were thereupon restored to favor, but neither has anyone ever discovered how these 'tomato sarcomas' were produced." A close second in charm of style is the occasional side remark revealing the philosophy of the author. At the end of a discussion of experiments and theories on page 16: "Ideas have arisen, tenuous at first, later taking form, to crystallize at last in a number of definite conceptions. Not yet having been consecrated by experiment, these remain only working hypotheses, but even so they have been immensely valuable. If some inaccuracies, and even errors, must be admitted, all hold a

germ of truth nevertheless, but their highest merit is that they stimulate research and bring about the clash of ideas that underlies all progress.'' Again in summary of our knowledge of cancer: ''We have the uneasy sensation that we know at once too much and too little.''

It is in the last chapter that the book becomes personal and the innermost thoughts of a coldly logical scientist seeking to harmonize the facts are clearly expounded. Those who look with favor on viruses as a cause of cancer will applaud, and those who still ignore many experimental observations will find their own arguments severely but fairly answered. How can one but admire an author who outlines the pros and cons, gives his own opinion in definite terms, and then writes: ''The only serious objection to be brought against the virus hypothesis at the present time is that it remains unproved.''

Oberling has something to contribute to the lay person, to the physician, to the investigator, and to the oncologist. To all, there is a brief stimulating discussion of cancer with emphasis on modern experimental studies. For the oncologist, even if he disagrees with the conclusions, there is an excellent bibliography.

ROBERT A. MOORE, M.D.

Fundamentals of Psychiatry. By Edward A. Strecker, M.D., Sc.D., F.A.C.P., Professor of Psychiatry and Chairman of the Department, Undergraduate School of Medicine, University of Pennsylvania; Psychiatrist to the Pennsylvania Hospital; Attending Psychiatrist, Psychopathic Division, Philadelphia General Hospital; Consultant to the Bureau of Medicine and Surgery, United States Navy; Consultant to the Secretary of War, A.A.F. J. B. Lippincott Company, Philadelphia, Pa., 1944, Second Edition. Price $3.00. Cloth with 219 pages and 15 illustrations.

This brief outline of psychiatry is presented by one of the leading teachers and practitioners of psychiatry. On the whole the author has been fairly successful in summarizing psychiatry for the student and general practitioner. The psychoses are presented quite clearly. His description of the psychoneuroses suffers from oversimplification and it is doubtful if the student will be able to obtain a clear picture of these conditions.

From the teacher's point of view this book has a number of defects. Although there is a brief historical introduction, no recognition is given to the men (Kraepelin, Freud, Janet Jung) who have contributed to the development of modern psychiatry. This deficiency could be remedied by the addition of one to two pages to the text. Recognition is given to Dr. William A. White but the name Kretschmer does not appear—this even in the section where two pages are devoted to types of physique. The same may be said for the conceptions of extrovert and introvert and their association with the name of Jung. The few references that are given in footnotes are to abstracts of books and articles and practically none to the original works, with the exception of a limited number of the author's own publications. It would seem a mistake to encourage students to rely on abstracts of the literature as a source of their knowledge.

The author devotes considerable space to the therapy of general paresis. This seems unwise because the present book constitutes only an introduction

to psychiatry and should not attempt to make the student depend on it as a therapeutic guide. On page 32 the author states that in the case of paresis, "The serology alone is so diagnostically decisive, that in an untreated case, the diagnosis may be made in the laboratory without seeing the patient." This statement encourages a student to rely too exclusively on laboratory techniques.

In spite of these limitations this introduction to psychiatry is one of the most useful outlines, available.

EDWIN F. GILDEA, M.D.

Homicide Investigation. LeMoyne Snyder, M.D., Medicolegal Director, Michigan State Police. Charles C Thomas, Springfield, Ill., 1944. Price $5.00. Cloth with 287 pages.

Written primarily for coroners and police officers, this book has much to recommend it as a practical reference and guide to physicians who occasionally participate in the investigation of suspected homicide. Excellently printed and well illustrated, the book covers both general and specific considerations of the medical and nonmedical phases of investigation.

Special chapters on the interrogation of the criminal, investigation of highway accidents, and the collection and preservation of firearms evidence were contributed by other members of the Michigan State Police and the Chicago Scientific Crime Detection Laboratory. The remaining chapters deal with examination of the scene, examination of blood stains, the detection of alcohol, examination for suspected assault, deaths from asphyxia, drowning, burning, poisoning, blunt and penetrating violence, criminal abortion, and gunshot wounds.

Appearing about the time when the "Report of the Committee of the American Medical Association to Study the Relationship of Medicine and Law" was published in the *Journal of the American Medical Association* (**125**: 577-583, June 24, 1944), it becomes obvious that there is a definite need of more general interest on the part of the medical profession for coordinated medical and legal activity. The author, who is also a member of the American Bar Association, is competent to present his views to both public officials and physicians. Exasperated at being confronted frequently in his work as a consultant by inadequate and misdirected investigation on the part of those charged with collecting the necessary preliminary evidence, Dr. Snyder has attempted to provide coroners, police officers, and others with a useful, simple handbook to serve as a pattern for proper investigation.

He has succeeded well in his purpose. The book is written for the most part in nontechnical terms. It shares the fault of any book that aspires to simplification; experts in the field will find numerous general conclusions that need qualification, but this does not detract from the value of the book. Laboratory procedures are referred to in general terms indicating the type of information which may be expected so that the coroner or police officer will not be tempted to carry out procedures beyond his scope.

The book includes over a hundred photographic illustrations, many of which are excellent. The quality of the reproductions of the color photographs is perhaps out of proportion to the added expense.

HERBERT S. BREYFOGLE.

The Diabetic Life, Its Control by Diet and Insulin. By R. D. Lawrence, M.A., M.D., F.R.C.P. (London), Physician in Charge, Diabetic Department, King's College Hospital, Late Chemical Pathologist and Lecturer, King's College Hospital. 13th Edition, Cloth with 228 pages and 18 illustrations. Price $4.00. The Blakiston Company, Philadelphia, Pa.

Lawrence is a well-known specialist in the treatment of diabetes, and this little book summarizes what he has found to be important and practical in the treatment of the disease. The information given is sound, and the directions are explicit. It is what it purports to be, "a concise practical manual for practitioners and patients." Since the first edition, published in 1925, the methods of this London physician have been widely followed in Great Britain, and have also been used by many people in this country. A "War-Time Supplement" heads the new edition, with special instructions and diets for war-torn England. For example, the proper use of the extra protein and fat rations granted to diabetics is outlined. Because of the scarcity of oranges, apples, tomatoes, and lettuce (all diabetic stand-bys), cabbage, turnips, carrots, celery, leeks, and watercress are recommended, and other similar practical substitutions are described. Lawrence's "Line-Ration" scheme of food measurement has always seemed to add to the complication of diet calculation. He seems to be abandoning it in favor of a "Five-Gram" diet scheme. American patients and physicians will no doubt continue to use the simpler carbohydrate-content food-group plan, and the food tables given in this book will not, therefore, be useful to the majority of patients and physicians in the United States.

Physicians interested in the treatment of diabetes will profit from the practical discussion of details of management given, but because of the difference in the American scheme of carbohydrate computation in the diet, other manuals available will prove of greater usefulness to diabetic patients in this county.

CYRIL M. MACBRYDE.

Elimination Diets and the Patient's Allergies: A Handbook of Allergy. By Albert H. Rowe, M.D., Lecturer in Medicine, University of California Medical School, San Francisco, California; Consultant in Allergic Diseases, Alameda County Hospital, Oakland, California. Lea and Febiger, Philadelphia, 1944, Second Edition, thoroughly revised. Price $3.50. Cloth with 256 pages.

The author of the book is a notable pioneer in the study of food allergy. He has made great contributions to the field of allergy, especially in the use of trial diets for diagnostic and therapeutic aids.

The book covers many symptoms and manifestations of allergy and attempts to correlate them with dietetic control. The main theme of the book is the dietary management of allergy. Rowe emphasizes, as he has done in previous books, the fallibility of skin tests, especially when chronic or delayed type of allergy is present. He very aptly stresses the importance of total exclusion of omitted foods while food allergy is being studied since it must be assumed that maximum food allergy is present. This practical point is overlooked by many who feel that a small amount of a forbidden food will do no

harm. The need of using a trial diet for more than a few days is emphasized, particularly in patients who have allergy of long standing.

Dr. Rowe expresses the fact, well known among allergists, that a period of observation must be long enough to determine whether freedom from symptoms is due to the use of a given diet or to an ordinary remission. He feels that a diet, to be of proved value, should relieve the patient longer than the longest previous symptom-free period. He stresses the necessity of detailed menus to assure proper nutrition during trial diet periods. He gives many standardized menus and mentions the difficulty of securing some of the suggested foods during wartime. Although the attempt is made to standardize trial diet usage, he does feel that a given diet should be modified by definitely positive skin reactions as well as the patient's history of disturbance from a given food.

The book is easily read. Although it discusses various etiologic agents and the clinical manifestations of allergy, its chief value lies in the emphasis it places on food in the etiology and therapeusis of allergy. The book should be studied carefully by all who practice allergy.

C. MALONE STROUD.

Principles and Practices of Inhalational Therapy. By Alvan L. Barach, M.D., Associate Professor of Clinical Medicine, Columbia College of Physicians and Surgeons; Assistant Attending Physician, Presbyterian Hospital. J. B. Lippincott Company, Philadelphia, Pa. Price $4.00. Cloth with 315 pages and 59 illustrations.

Dr. Barach qualified himself as the author of this handbook by devoting years of study analyzing the techniques and rationale of inhalation therapy. He has done an admirable job of summarizing his own observations and those of other investigators in a thoroughly readable form. After presenting the historical and physiologic background of inhalation therapy, he systematically discusses its use in the treatment of thirty different conditions. A few of these are acute altitude sickness, pneumonia, pulmonary edema, congestive heart failure, shock, coronary thrombosis, emphysema, bronchial asthma, war gas poisoning, and blast injuries of the lungs. Each section begins with a statement of the pathologic physiology of the disease state and an analysis of the rationale for using inhalation therapy. There is a valuable chapter on oxygen poisoning which calls attention to the apparent ability of human subjects to withstand higher concentrations of oxygen than those tolerated by most experimental animals. Barach suggests that the greater tolerance may be due to the fact that patients who breath high concentrations of oxygen have intermissions during which the mask is changed or their faces washed. The detailed discussion of methods of inhalation therapy contains much practical information as, for instance, the necessary minute flow of oxygen to attain different concentrations of the gas in inspired air, when face masks are used.

The book is beautifully printed, well illustrated, adequately indexed, and contains a good bibliography. As a minor criticism, it should be pointed out that many fundamental investigations done on animals are reviewed only very briefly. The author avoids, however, the mistake of being too enthusiastic in his claims for the possible benefits of inhalation therapy.

C. V. M.

The Analytical Chemistry of Industrial Poisons, Hazards and Solvents. By Morris B. Jacobs, Ph.D., Senior Chemist, Department of Health, City of New York, 1928-; Director of Gas Reconnaissance, Gas Defense Service, New York City Citizens Defense Corps; formerly Lt. U. S. Chemical Warfare Service Reserve. Second revised reprint. 1944, Interscience Publishers, Inc., New York, N. Y. Price $7.00. Cloth with 661 pages.

The Diet Therapy of Disease. By Dr. Louis Pelner, Assistant Attending Physician, Long Island College Hospital, Greenpoint Hospital, and Brooklyn Cancer Institute; Gastroscopist; Beth Moses Hospital; Lecturer, Postgraduate Course in Gastroenterology, under the charge of Dr. S. A. Seley. Personal Diet Service, New York, N. Y., 1944. Price $3.75. Cloth with 143 pages.

A Manual of Physical Therapy. By Richard Kovacs, M.D., Professor of Physical Therapy, New York Polyclinic Medical School and Hospital; Attending Physical Therapist, Manhattan State, Harlem Valley State, Columbus, and West Side Hospitals; Visiting Physical Therapist, New York City Department of Correction Hospitals; Consulting Physical Therapist, New York Infirmary for Women and Children; Mary Immaculate Hospital, Jamaica, New York, St. Charles Hospital, Jefferson, L. I., Hackensack Hospital, Hackensack, N. J. Third edition, thoroughly revised, formerly published under the title "Physical Therapy for Nurses" Lea & Febiger, Philadelphia, Pa., 1944. Price $3.25. Cloth with 309 pages and 118 engravings.

The Principles and Practice of Medicine. By Henry A. Christian, A.M., M.D., LL.D. (Hon.) Sc.D., Hon. F.R.C.P. (Can.), F.A.C.P., Hersey Professor of the Theory and Practice of Physic, Emeritus, Harvard University; Clinical Professor of Medicine, Tufts College Medical School; Physician in Chief, Emeritus, Peter Bent Brigham Hospital; Visiting Physician, Beth Israel Hospital, Boston. Fifteenth edition, D. Appleton-Century Co., New York, N. Y. Price $9.50. Cloth with 1,498 pages.

The Treatment of Peptic Ulcer. By George J. Heuer, M.D., Professor of Surgery of Cornell University Medical College and Surgeon-in-Chief of the New York Hospital. J. B. Lippincott Company, Philadelphia, Pa. Price $3.00. Cloth with 118 pages.

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